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THE TRANSMISSION OF "LOUPING-ILL" TO THE MOUSE AND THE MONKEY: HISTOLOGY OF THE EXPERIMENTAL DISEASE.

By E. WESTON HURST.

Milbank Research Fund, Bacteriological Department, Lister Institute, London.

IN 1930, Pool, Brownlee and Wilson showed that some cases of the clinical entity in sheep known as "louping-ill" were due to infection with a filtrable virus of neurotropic tendencies; experimentally the disease could be transmitted to the sheep and the pig. Greig, Brownlee, Wilson and Gordon (1931) briefly designated the lesions a meningo-encephalo-myelitis but furnished no detailed information. They transmitted the malady to mice and with less certainty to rats; the rabbit and the guinea-pig proved refractory. Alston and Gibson (1931) confirmed the possibility of transmission to mice but failed to infect rats, guinea-pigs, and rabbits. In contradistinction to the preceding authors, they obtained in mice evidence of direct case-to-case infection.

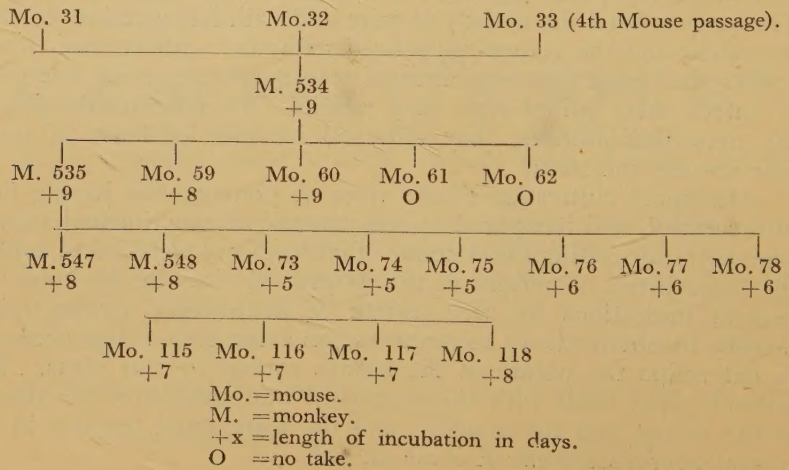
Attempted cultivation of the virus of poliomyelitis having been unsuccessful, and hampered by the expense of experimental animals (monkeys) for testing the potency of cultures, we were led to explore the possibilities of culture of the louping-ill virus in the hope that general indications for the growth of neurotropic viruses might thereby be obtained. In consequence, as a first step, it was necessary to determine the nature of the lesions caused by this virus. The present paper deals with the successful transmission of the disease to the mouse and the monkey, and the histological features in the central nervous system of these animals.

Experimental Work.

The infective material, kindly supplied by Mr. W. A. Pool, was sheep brain representing the second passage of the brain from a spontaneous case. A 1 per cent. emulsion in hormone broth was filtered through an English Berkefeld candle and tested for sterility. Inoculated intracerebrally in doses of about 0·05 c.c., it produced typical symptoms in five out of eight mice, with incubation periods of from six to 14 days. Thereafter, the virus was passed intracerebrally through eight generations of mice without a single failure to develop the disease ; in these the incubation period was nearly always five or six days, occasionally four or seven. Unfiltered brain-emulsions, whether of homologous or heterologous brain, appeared to be much more toxic than filtrates and led to the immediate death of a definite proportion of the animals receiving them. All mice tested were susceptible independent of their colour.

Attempted transmission to four young rabbits and six adult rats failed.

From the pooled brains of the fourth generation of mice a 5 per cent. emulsion was prepared, and 1·0 c.c. inoculated intracerebrally into a healthy monkey (M. 534—*M. rhesus*). Nine days later the animal developed symptoms to be described presently, and was killed when moribund three days later. The brain was passed into another monkey (M. 535), which developed, again on the ninth day, symptoms identical with those in the first. The brain of M. 535 produced after an incubation period of eight days exactly similar symptoms in two further animals (M. 547 and 548), thus bringing the disease in the monkey to the third generation. At each passage the monkey brain was capable of inducing the typical disease in mice, though at the first and the third after a rather longer incubation than normal ; this is shown in the accompanying diagram. Single attempts to infect monkeys with the brain of M. 548 inoculated intramuscularly or into the sciatic nerve failed.



Clinical Features.

Symptoms in the Mouse.

Early symptoms are rather variable. The mouse may be quiet and apathetic, sitting hunched up with the hair raised. Or general hyperæsthesia may be evident, when the mouse jumps violently at a slight noise or light touch. A fine tremor may be present at rest and a coarse tremor or gross ataxia on movement. Some mice show a curious jerky progression recalling a flickering cinematograph film ; others are merely slow, stiff and awkward in movement, or, again, tend to walk in circles. Muscle spasms may occur.

Paresis and inco-ordination of the legs, at first of paraplegic or hemiplegic distribution and spreading later to the other limbs, may progress to complete paralysis in the hindlimbs ; even at death, however, some power usually persists in the forelimbs. No rigidity has been observed. Dribbling of urine, associated in some cases with a greatly distended bladder, is common.

The animal finally passes into a comatose condition in which it does not respond readily or at all to stimuli ; breathing is slow and laboured and death supervenes.

In a number of mice the disease has been of extremely rapid development, and a mouse apparently normal or with only some tremors one evening has been found dead next morning with typical histological changes in the central nervous system. Except in two instances, when the course of the disease was protracted to three days, no animal has survived more than two days after the onset

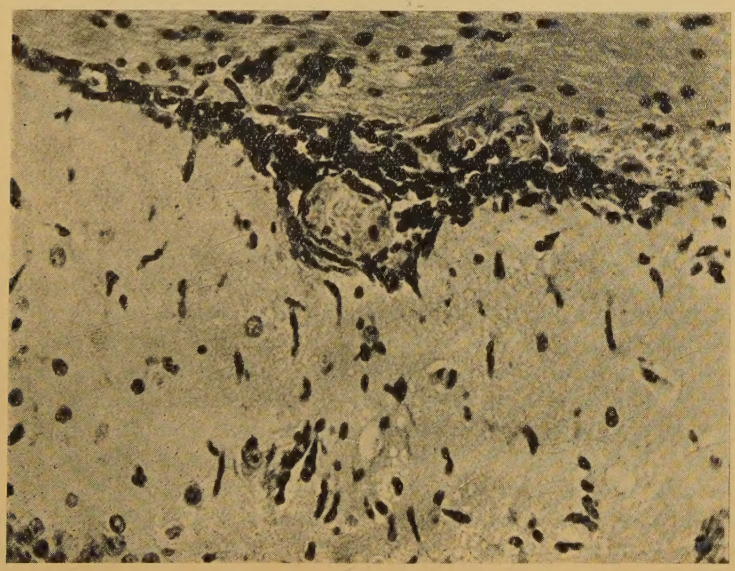


Fig 1.

Mouse. Meningeal infiltration with rod-cell reaction in underlying tissues.

of the first symptom. All animals used for detailed histological study were killed when moribund, or at an earlier stage.

Symptoms in the Monkey.

Here the clinical picture differs considerably from that in the mouse and, judging from experience with four animals, is quite characteristic in its stereotypy. In brief, the symptoms are those of a pure ataxia of cerebellar type.

The first sign of disease is slight clumsiness and unsteadiness on attempted movement, especially sudden movement, and the monkey bumps into obstacles or against the side of the cage. In the course of twenty-four hours ataxia increases until the animal is unable

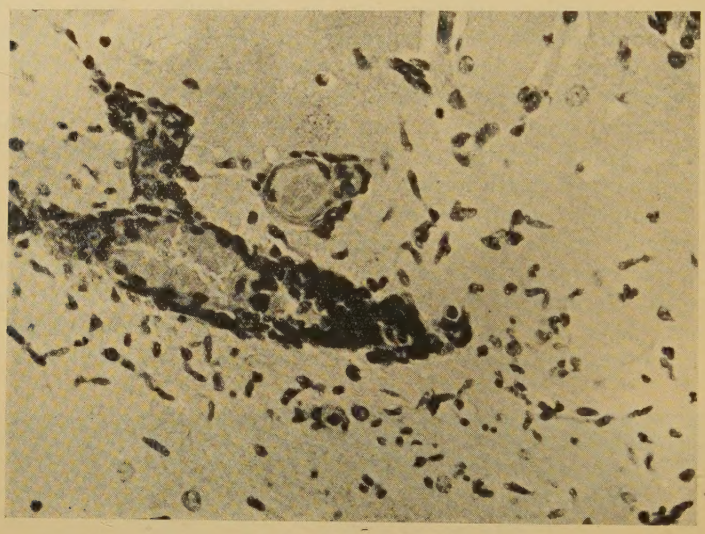


FIG. 2.
Mouse. Perivascular infiltration with microglial (largely rod-cell) reaction in nervous tissues.

to walk upright without falling; it can still move erratically on all fours. (In one case, M. 547, the animal remained in the slightly unsteady state for five days, after which symptoms rapidly progressed.) By clinging to the bars of its cage it raises itself slowly and shakily to a sitting position and maintains this posture for hours together; efforts to displace it are resisted, and during the attempt the head swings wildly and comes into frequent and violent contact with the cage. Nystagmoid movements of the eyes are present. In the last stages progression of any kind is impossible, yet even now muscular power is considerable and no sensory loss can be determined. Tendon reflexes are retained. Visual perception appears normal.

The temperature, which on the seventh or eighth days may show a rise of from 1° to 2° F., falls progressively with the appearance of

nervous symptoms. The animals were killed when moribund with a subnormal temperature on the third, third, fourth, and eighth days respectively of the developed disease.

Histology.

The brains of eighteen mice, together with those of nine controls and four monkeys, were examined fully by neuropathological methods. In none of the controls were spontaneous lesions present, though in a later batch of mice encephalitozoon infection was recognised.

Lesions in the Mouse.

In a general way lesions bear a resemblance to those obtaining in the rabbit infected with a street rabies virus. Their appearance coincides in time with the development of tremor and hyper-excitability, when early lesions may be found in all parts of the central nervous system.

Meningeal infiltration with lymphocytes, large and small, a few large mononuclears and in some cases a few polymorphs is only sometimes intense and may be wanting almost wholly, particularly over the brain-stem and cord. It is most obvious in the main fissures of the cerebrum, around the larger vessels, and at the base. Pyknosis and karyorrhexis are commonly seen in the infiltrating cells.

In the nervous tissues proper, many of the vessels are cuffed with one or several layers of similar cells, in some of which numerous fine fatty granules are present. Around the vessels and over areas

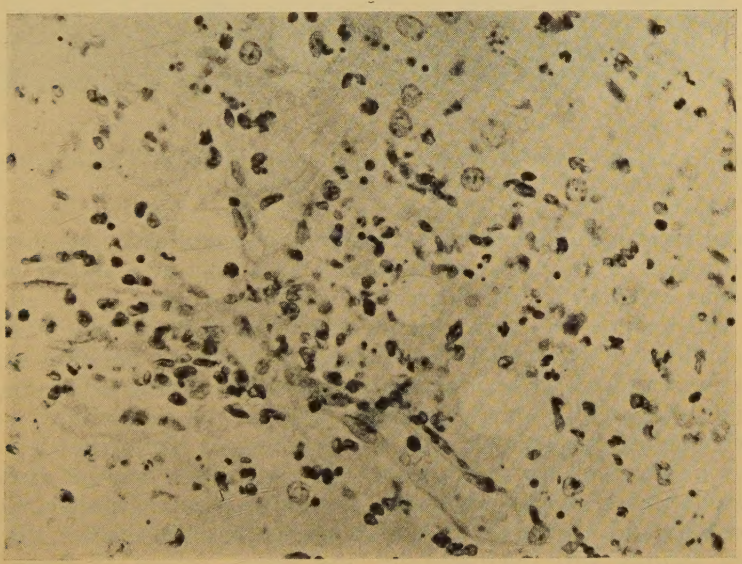


Fig. 3.

Mouse. Microglial and polymorphonuclear infiltration of nervous tissues. Pyknosis and fragmentation of nuclei of many infiltrating cells.

of cerebral tissue microglial proliferation is evident, and in extreme cases may affect the whole cross-section of the cerebrum ; focal collections of more densly packed cells occur at intervals, both in association with, and independently of such diffuse infiltration. At an early stage these cells nearly all take the form of very long " stäbchenzellen " ; later they are highly polymorphic and the nuclei of some show karyorrhexis. In a few cases they contain numerous fine fatty granules. A few lymphocytes or polymorphs may take part in the tissue infiltration ; the latter undergo rapid degeneration and contribute to the pyknotic globules scattered around.

As seen in Nissl preparations the affected nerve cells manifest a great variety of pathological changes. Partial or complete

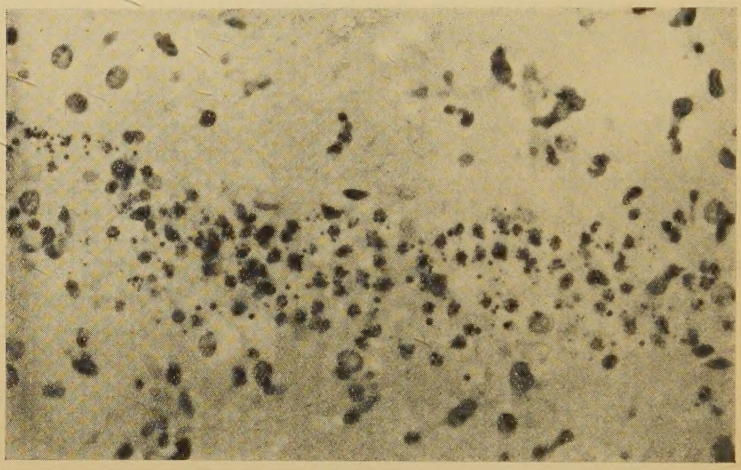


FIG. 4.
Mouse. Massive acute necrosis in cornu Ammonis. Nuclear fragmentation in necrotic nerve cells.

chromatolysis combined with moderate or great swelling gives all appearances from a coarsely granular to a pale and almost homogeneous cytoplasm. All degrees of vacuolation may be noted, and the cell-margin sometimes shows large indentations with sharply-cut outlines. In the most damaged cells coarse basophil masses may be scattered on or immediately outside a fading nuclear membrane. Frequently, in the granular layer of the cerebellum, a high percentage of the granule cells are pyknotic or karyorrhectic. The large neurons of the brain-stem and cord often exhibit irregular hyperchromasia of the cytoplasm, from which the shrunken, hyperchromatic and excentric nucleus is hardly distinguishable. Finally, when stained with hæmatoxylin and eosin, others of these cells have a bright pink cytoplasm and a fading fragmented nucleus recalling the picture of acute nerve-cell necrosis seen in monkey poliomyelitis. As was the case with other silver methods, in the

mouse Bielschowsky preparations were not uniformly successful. In the early stages the neurofibrils of the motor cells of the cord are well-preserved ; later, a granular picture or no staining at all is achieved.

Neuronophagia of damaged neurons by the microglia is not infrequent, especially in the cord. By the third day appearances very like those in poliomyelitis are present, and foci of microglial phagocytes, surrounding perhaps masses of structureless debris, alone indicate the site of vanished nerve cells.

In cases examined by the Weigert-Pal method no myelin degeneration has been noted.

Though the most extreme nerve-cell changes are encountered in the later stages (*e.g.*, on the third day), from the beginning some degeneration is evident ; moreover, their distribution is not necessarily closely dependent on that of the vascular and interstitial phenomena. These observations suggest a direct attack by the virus on the functional nervous elements.

Distribution of Lesions.

Perivascular and tissue infiltration are nearly always more marked in the cerebrum, where the *basal nuclei* and all areas of the *cortex*, including the cornu Ammonis, are about equally affected ; in the *brain-stem* and *cord* both are more variable. The *cerebellum* may be severely damaged or almost normal ; the mouse does not show the constant and outstanding involvement of this organ seen in the monkey. Nerve-cell degeneration also is present at all levels, but the more severe grades are found chiefly among the large neurons of the brain-stem and cord. In one animal dying on the third day, however, not only are the majority of these cells missing, but there is massive acute necrosis in the cornu Ammonis and in the Purkinje-cell layer of the cerebellum. In the single animal in which the *spinal* and *gasserian ganglia* have been examined no lesions can be detected ; in several, however, the ganglia lying between the adrenal and kidney show considerable interstitial infiltration with mononuclears and polymorphs, together with degenerative changes in some of the nerve cells.

Inclusions in the Nerve Cells.

Observation of Nissl sections at an early stage of the disease reveals the presence in the nerve cells of small round or oval clear areas surrounded by purplish rings of irregular thickness, often prolonged into little tails or angles. The clear areas gradually enlarge and lose their purple envelope. Other stains demonstrate tinctorial properties not shown by the protoplasm and it is difficult to believe that the bodies do not belong to the general class of cytoplasmic inclusions.

Invariably multiple and often very numerous, they are commonly of oval shape and attain a diameter of from one to six or eight μ. At a late stage larger kidney-shaped, rod-like or irregular masses may be

found in the large neurons of brain-stem and cord. With methyl-blue-eosin after sublimate-formol fixation they colour a bright red, slightly less vermillion than the red blood-corpuscles and distinctly less crimson than the nucleoli ; their tint is always brighter in the hyperchromatic nerve cells. The smaller round or oval forms show no definite structure ; the larger irregular bodies exhibit a structure such as might result from the irregular deposition of stainable substance around several centres. In some of the large forms small uncoloured spaces may be seen. With Campanacci's method their colour is deep red. By Giemsa's method only a very faint pink shade is obtained, and though by prolonged staining in phloxin (followed by methylene blue) they appear a definite pink, neither method is as effective for their demonstration as the preceding. By none of these methods nor by Stützer's method or by silver impregnation can definite inner bodies be demonstrated, and the irregular shape of the large bodies further differentiates them from Negri bodies. At a certain stage of decolorisation after iron alum-hæmatoxylin, when many of the bodies are completely decolourised and others still black throughout, a few may show a central round, oval or rod-shaped black structure ; the results of decolourisation for different lengths of time suggest that this appearance may merely indicate progressive bleaching of the bodies from the surface and not a true inner formation.

The bodies are probably always intracytoplasmic ; since they continue far into the main processes of the cell, those apparently lying free between the nerve cells may well be contained in axons or dendrites originating from cells at a distance. The cells containing them are not necessarily much altered from the normal. They have never been seen in the nuclei of the nerve cells or in glial or vascular elements. They are most numerous in the neurons of the brain-stem and cord, and especially in the *nuclei pontis* where practically every cell contains a shower. The Purkinje cells sometimes contain numerous fine bodies, and bodies are fairly abundant in the basal nuclei. In the cortieal neurons they are met with less frequently, and in fewer numbers ; here they are never of large size. The cornu Ammonis is no richer in inclusions than the general cortex. Bodies may also be seen in the nerve cells of the ganglia lying between the adrenal and kidney. The bodies survive for some time the death of the cells containing them and are recognisable in masses of cellular debris undergoing neuronophagia.

The apparent mode of development of these structures is of considerable interest, and the appearances in Nissl sections has already been detailed. At an earlier stage, in some of the large motor cells of the brain-stem and cord, their distribution is exactly similar to that of the Nissl bodies ; with methylblue-eosin they colour a much paler pink, and the encircling zone of blue material is wider and its outlines that of a mass of tigroid substance. Still earlier, Nissl bodies of characteristic shape and distribution may show a paler central area taking a very faint pink tinge. This suggests that

early stage in the formation of the bodies may coincide with some change, perhaps specific, in the precipitable chromophilic constituent of the nerve-cell cytoplasm. That the problem is not straightforward, however, is apparent when it is remembered that the Nissl substance exists during life as a fluid cellular constituent of the cell which is precipitated in characteristic form only on application of the fixative. Moreover, in other cells the bodies appear first as tiny granules and they may be seen in cells containing normally no Nissl bodies. The fact remains, however, that when first developed the inclusions are often not nearly so strongly acidophil as at an advanced stage, and that, perhaps for mechanical reasons, they may then show a close association with the masses of tigroid substance.

Although clumps of tiny red granules may sometimes be seen in the normal nerve cells of the mouse, and the chromophilic cells

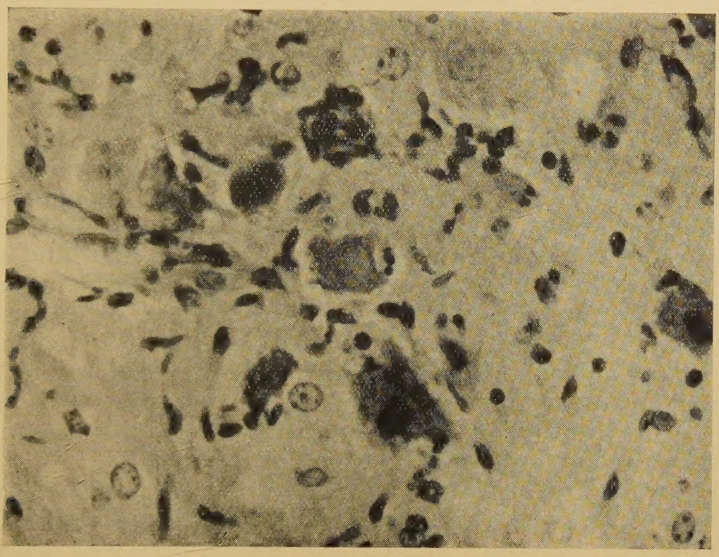


FIG. 5.

Mouse. Degeneration and neuronophagia of anterior-horn cells of spinal cord with microglial reaction in nervous tissue.

may sometimes take with methylblue-eosin a diffuse purplish-red tinge, the appearances described have never been seen in control animals, or in mice infected with encephalitozoa.

Lesions in Other Organs.

With the following exceptions no lesions not present in one or other of the control mice were detected in the other viscera.

Several animals showed marked congestion of the *lungs*, and two, terminal hæmorrhage into the alveoli.

One animal with a greatly distended *bladder* showed a large recent hæmorrhage lying between the layer of subepithelial fibrous tissue and the muscle coat.

Lesions in the Monkey.

Here the constant change appears to be the striking affection of the cerebellum, providing a complete explanation of the symptomatology ; elsewhere lesions though widespread are of variable severity and distribution from case to case.

Meningeal infiltration is insignificant and is restricted to small groups of mononuclear cells around some of the larger vessels of the cerebral hemisphere and brain-stem.

One case shows considerable alterations in the *cerebral cortex* ; in three lesions are much less marked. They consist in perivascular cuffing of a variable number of the vessels, often accompanied by some microglial proliferation in the surrounding tissues. The infiltrating cells are largely lymphocytes with a few plasmacytoid and plasma cells and, very rarely, an occasional polymorph. Small compact microglial foci occur among the nerve cells, less often in the superficial zone immediately under the meninges or in the subcortical white matter. Where lesions are severe, larger areas of more diffuse microglial proliferation show also a few lymphocytes, occasional plasma cells and very rare polymorphs in the nervous tissues. Many of the nerve cells are well preserved, a few are vacuolated, and some, particularly the Betz cells, have suffered central or complete chromatolysis. In one case, some of the cortical neurons are surrounded by large numbers of satellites ; a few are severely damaged and undergoing neuronophagia. In different cases these alterations are more marked in the anterior or in the central regions ; in none is the occipital cortex involved to an appreciable extent. The cornu Ammonis is affected in only one case.

In the brain with most severe cortical damage, similar changes are equally pronounced in the *caudate* and *lenticular nuclei*, but not in the *optic thalamus* ; in the *globus pallidus* nerve-cell degeneration is frequently advanced, and many neurons have succumbed and are in process of neuronophagia. In the remaining cases the *optic thalamus* and tissues around the *third ventricle* have borne the brunt of the attack in the basal regions.

In the monkey with most cortical involvement, the *brain-stem* and *cord* are scarcely affected ; in the others, lesions are more severe in the brain-stem than in the cerebral hemisphere and attain their maximum development in the *pons*. In one animal the picture closely resembles that seen in monkey poliomyelitis.

There is intense perivascular cuffing with large and small lymphocytes and occasional plasma cells and large mononuclears. Microglial proliferation, both focal and diffuse, is very marked in the dorsal half of the organ and extends by way of the peduncles as far as the dentate nuclei ; lymphocytes and a few plasma cells are also present

in the nervous tissues. Pyknosis and karyorrhexis are frequent among the infiltrating cells. While a few of the nerve cells are normal, others show marked degenerative changes. In Nissl preparations many exhibit great swelling of the body with complete chromatolysis and corresponding nuclear alterations ; in addition, vacuolation and investment by microglial phagocytes may be evident. With hæmatoxylin and eosin a few cells are seen to have suffered acute necrosis and, normal in size and shape, present a brightly eosinophil body and a fading fragmented nucleus. Neuronophagia is active, and many nerve cells have already been removed completely. Bielschowsky impregnation of the more damaged cells often fails to reveal a fibrillar apparatus, but in some cells the curious appearance is seen of a single, enormously thickened fibril entering the cell body from a process and terminating abruptly in a more or less complicated spiral or loop. In one or two areas actual softening of the tissues seems imminent.

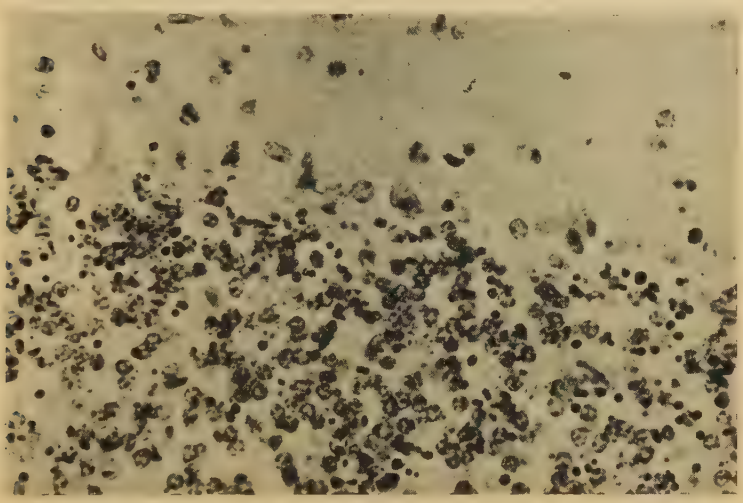


FIG. 6.
Mouse. Cerebellum. Pyknosis of many of granular cells ; degeneration of Purkinje cells.

In the *spinal cord* similar changes in the grey matter vary greatly in intensity. While chromatolysis is frequent, more severe changes leading to death of the anterior horn cells are not very common, and acute necrosis is not seen ; the findings here are thus very different from those in poliomyelitis.

The cerebellum is the seat of remarkable lesions and is relatively far more severely affected than in other neurotropic virus diseases. In brief, there is wholesale destruction of the Purkinje cells, of which only a small proportion survive in recognisable condition ; microglial reaction is disproportionately meagre. Except in M. 547, where symptoms were of slower progression, and lesions, though severe,

are less marked than in the other monkeys, over wide stretches of the *cortex cerebelli* no Purkinje cells are seen ; occasionally a distorted necrotic fragment may be detected, but more often an empty cell-nest alone indicates the site previously occupied. Although in parts of the adjoining molecular layer moderate proliferation of microglial cells is evident, this is by no means always the case, and along the line of the Purkinje cells there is no massing of these cells as there is around necrotic neurons in the brain stem. A few leucocytes, lymphocytes and plasma-cells are present here, and there is some enlargement and proliferation of glia. The myelin sheaths are markedly varicose. Where Purkinje cells remain, some few are normal ; others exhibit irregularities of staining, shrinkage, vacuolation and

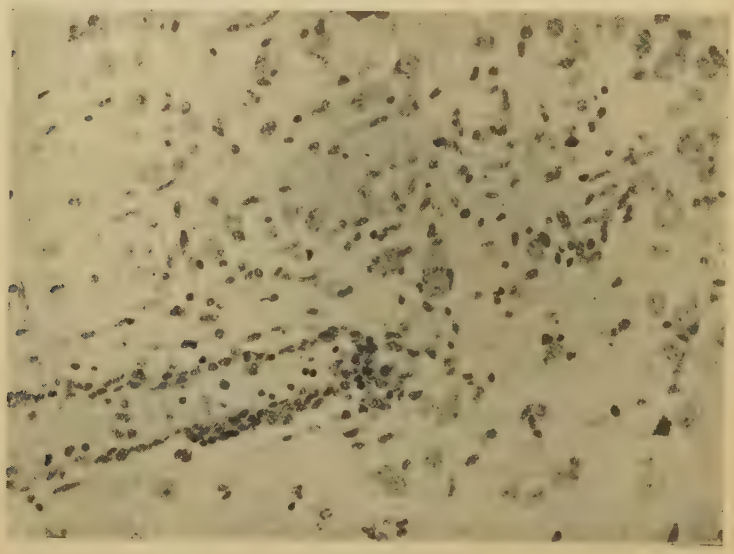


FIG. 7.
Monkey. Perivascular infiltration in cerebral cortex. Microglial reaction in nervous tissues. Satellitosis of neurons.

similar signs of degeneration. The condition of the *dentate nucleus* resembles that of the brain-stem.

In the *spinal* and *sympathetic ganglia* of the one case examined from this point of view, numerous mononuclear foci are present in the interstitial tissues and a few ganglion cells show degenerative changes or have disappeared entirely.

Inclusions.

Structures like those described in the mouse have not been observed. With methylblue-eosin isolated tiny red granules may be seen in the nerve cells in many regions, while in the brain stem, and particularly in the cells of the *nuclei pontis*, they are more numerous ; as similar granules have been found in equal numbers in many

controls they are evidently of no significance. The existence of numerous tiny eosinophil granules in some of the spinal ganglion cells of man and monkey has long been recognised.

Other Organs.

No lesions not present in controls have been discovered.

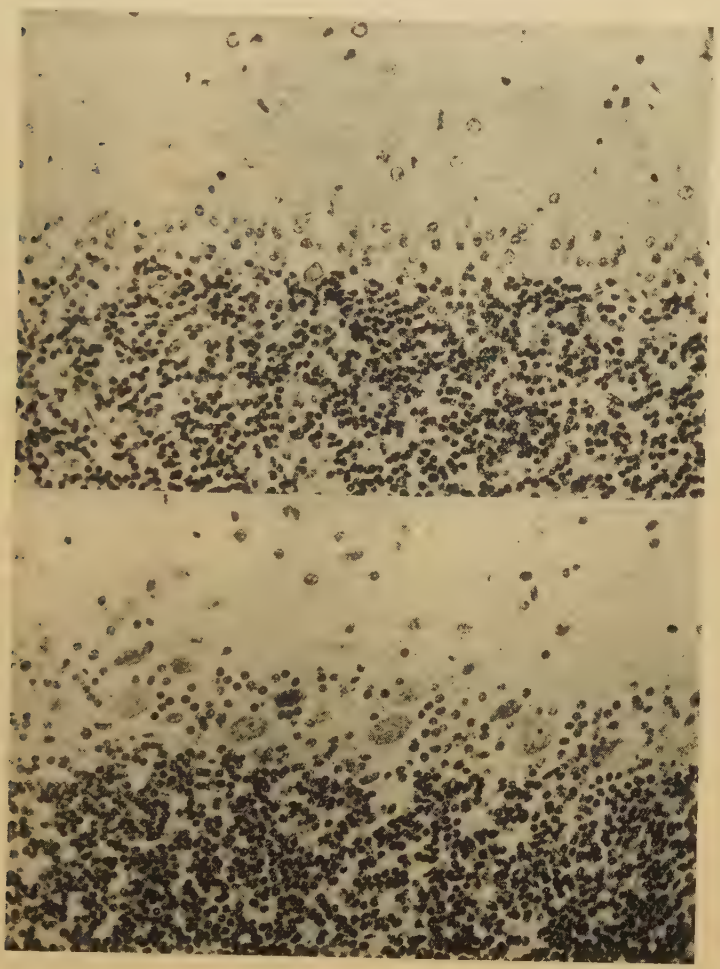


FIG. 8.

Monkey. (*Above*) Normal cerebellum. (*Below*) Cerebellum of animal with louping-ill, showing disappearance of Purkinje cells.

COMMENT.

Intracerebral inoculation of the virus of "louping-ill" is thus productive in both the mouse and the monkey of a definite encephalomyelitis which, while partaking of the general characteristics of

neurotropic virus infections, possesses certain features peculiar to itself. In this light we may probably regard the peculiar intracytoplasmic structures which seem to be specific for the disease in the mouse, while the especial involvement of the cerebellum in the monkey affords at once an analogy to the extraordinary selective action of the virus of poliomyelitis on the anterior-horn cells of the spinal cord, and a ready means of distinguishing the present disease, both clinically and pathologically, from other forms of encephalomyelitis in that animal. Meningeal involvement is obviously of secondary importance, and the main incidence of the virus assault is on the nervous tissues. Certain observations in the mouse suggest a direct attack by the virus on the nerve cells, which do not, apparently, suffer only in consequence of the inflammatory reaction taking place in their vicinity.

The absence of the bodies which have been accepted as intracytoplasmic inclusions in the mouse from the tissues of the monkey is of great interest. The experimental results already detailed leave no doubt that the disease in the monkey was " louping-ill," yet, histologically, the formation in the nerve cells of the peculiar eosinophil masses so frequent in mice was never observed. This, with the outstanding involvement of the cerebellum in the monkey, can only represent a difference in species-reaction to one and the same infective agent.

Summary.

1. The disease known as " louping-ill " is transmissible to the mouse and to the monkey.

2. In the mouse, among the rather variable symptoms, paralysis and inco-ordination take a prominent place. The lesions are of the nature of a diffuse encephalomyelitis with mild meningeal involvement.

3. In four monkeys, a progressive ataxia of cerebellar type was the dominant clinical feature. Histologically a more or less diffuse encephalomyelitis is accompanied by massive destruction of the Purkinje cells of the cerebellum. The analogy to the destruction of the anterior-horn cells in poliomyelitis is drawn.

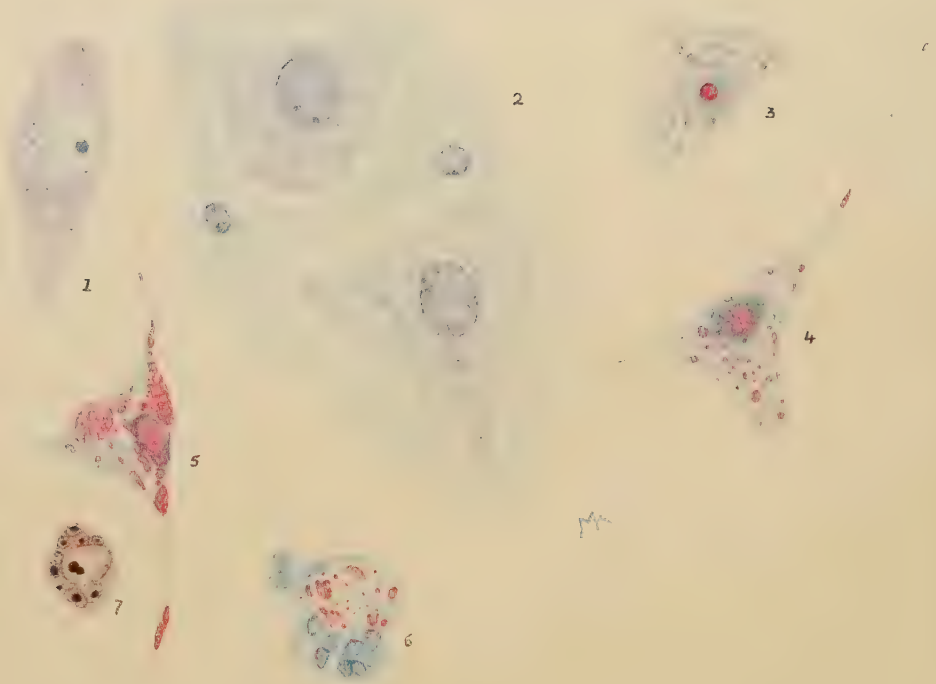
4. In the mouse, but not in the monkey, intracytoplasmic bodies believed to be of the nature of inclusion bodies are found in the nerve cells.

References.

Alston, J. M., and Gibson, H. J. 1931. *Brit. J. Exp. Path.*, 12, 82.

Greig, J. R., Brownlee, A., Wilson, D. R., and Gordon, W.S. 1931. *Vet. Rec.*, 11, 325.

Pool, W. A., Brownlee, A., and Wilson, D. R. 1930. *J. Comp. Path. & Therap.*, 43, 253.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Description of Coloured Plate.

1. Cytoplasmic bodies in a nerve cell of a mouse.
   Formol-saline: Nissl.

2-5. Successive stages in development of the bodies.
   Sublimate-formol: Methylblue-eosin.

6. Cytoplasmic bodies in a neuron undergoing neuronophagia.
   Sublimate-formol: Methylblue-eosin.

7. Appearance after iron alum-hæmatoxylin and partial decolorisation with iron alum.
   Sublimate-formol: weak eosin counterstain.

OVINE AND BOVINE STRAINS OF B. CHAUVŒI.

By R. S. Roberts.

S.E. Agricultural College, Wye, Kent.

On searching the available literature on blackquarter infection of cattle and sheep an allusion is often found to the unaccountable fact that there is no evidence of wounding preceding cases of the disease in bovine animals, whereas the same disease usually occurs in sheep as a direct and obvious sequence of wounding, and this has led some authors to investigate the cultural, biochemical, and serological characters of the causal organism, *B. chauvœi*, with a view to determining whether differences exist between strains isolated from cattle and sheep. Zeissler (1924) carried out an extensive study of the agglutination reactions of the two organisms, but did not attempt to distinguish them by cross absorption of agglutinins. No differences were found, but the sera used appear to lack uniformity of titre, both for homologous and heterologous antigens. Zeller (1927) found it impossible to distinguish between the two. Jungherr (1928) carried out both agglutination and absorption tests and found no differences. Bosworth (1931) states that the two organisms are identical morphologically, culturally, and serologically. Nitt and Strickdorn (1919) and Zeller (1927) state that the two diseases have a different distribution, the two not occurring on the same pastures.

Offsetting the evidence pointing to the disease being caused by different types of *B. chauvœi* is the fact that some authors, including Viljoen and Scheuber (1927) and Marsh, Welsh and Jungherr (1928), report the ovine disease as occurring in an apparently spontaneous manner.

In a recent paper (Roberts and McEwen, 1931) an account was given of the detailed bacteriological examination of 29 cases of gas gangrene, from 25 of which, examined soon after death, *B. chauvœi* was isolated. Amongst these only one was, as far as could be ascertained, unconnected with wounding of any description. This occurred on pastures where wound gas gangrene caused by *B. chauvœi* is prevalent, and, moreover, in the same year *B. chauvœi* was isolated from the body of a heifer which, together with several others, had died of blackquarter. Thus, bovine blackquarter, spontaneous ovine blackquarter, and wound gas gangrene due to *B. chauvœi* have all been encountered on the same pastures.

A careful study was made of eleven of the strains of *B. chauvœi* recovered from sheep dead of gas gangrene, of the one from a spontaneous case of blackquarter in a sheep, and of the bovine strain; and culturally, morphologically, and biochemically all were identical and conformed to the classical reactions and characters of *B. chauvœi* of bovine origin. When the agglutination test was applied, using a

polyvalent serum made with four ovine strains, and suspensions of each strain, it was found that the suspension of bovine bacilli was agglutinated to a considerably lower titre than were those of the ovine strains. Similarly, it was found that a serum made with bovine bacilli also agglutinated suspensions of the eleven ovine strains to a far lower titre than its homologous suspension.

It was then decided to carry the investigation further, using a few typical strains and producing all sera in a strictly comparable manner.

Agglutination.

Three strains from cases of gas gangrene in sheep, one from a case of spontaneous blackquarter in a sheep, and three bovine strains were used. All these had previously been studied in detail as to their morphological, cultural, and biochemical characters, and had been found to conform to the general type. Stock cultures were sub-cultured into meat broth and these used to inoculate 250 c.c. quantities of diffusion shell glucose broth, which was then incubated for 18 hours, at which time the vegetative elements were in the process of metamorphosis into the navicular forms. The growth was collected, washed twice in normal saline solution, re-suspended to a standard opacity, and ·5 per cent. phenol was added. This suspension was used in the agglutination tests. 5 c.c. quantities of the same suspensions of each strain without phenol were inoculated intravenously four times on alternate days into each of seven rabbits, and serum was obtained seven days after the last inoculation. The normal sera of all the rabbits used were tested for natural agglutinins for both bovine and ovine suspensions and found negative. The rabbit immune serum of each bovine strain was tested for agglutinins for the four ovine strains and for one bovine suspension. Similarly, the three bovine strains were tested for agglutinins for all the ovine strains and for one bovine strain. In addition, the strain which was derived from the case of spontaneous gas gangrene in a sheep was tested against the bovine and the ovine homologous suspensions. The results (shown on page 250) give a striking indication of an antigenic difference between the bovine and ovine strains. The strain from the spontaneous case falls into the ovine group.

An analysis of the figures shows that bovine and ovine sera agglutinate homologous suspensions to a high titre, and the heterologous suspensions to a low titre.

Absorption of Agglutinins.

In order to establish the significance of the results obtained by cross agglutination, it was deemed necessary to ascertain to what extent the bovine and ovine strains absorbed agglutinins for the heterologous antigens, both from the homologous and heterologous sera. Absorption was carried out by leaving a known volume of serum in contact with ten times the quantity of suspended bacteria usually employed per tube in the direct agglutination test and incuba-

ting in air at 37° C. for two hours. The results obtained may be summarised as follows :—

1.—The bacterial suspensions used were not agglutinated specifically by normal rabbit serum.

2.—Cross-agglutination, using untreated sera and the same suspensions, proceeded in the characteristic manner already noted.

3.—Absorption of the ovine serum by ovine antigen deprived the serum of its agglutinins for the bovine antigen, although absorption was incomplete, as shown by the fact that agglutination of the homologous antigen has occurred to a low titre. Absorption of the same serum under equal conditions by the bovine suspensions removed all agglutinins for the bovine antigen but failed to remove any of those for the ovine antigen. Absorption of the ovine serum by bovine bacteria did not in fact materially alter the titre of the ovine serum for the ovine antigen.

4.—When the bovine serum is absorbed with bovine and ovine antigens the whole process is reciprocated. That is, the ovine suspension when used to absorb the bovine serum fails to alter materially the titre of the bovine serum for the bovine suspension.'

The results of cross-agglutination tests undoubtedly point to a community of antigens, but, as shown by absorption tests, neither the ovine nor the bovine suspension is capable of removing from the heterologous serum its agglutinins for its homologous antigens. It therefore appears that the two strains possess both a common and a specific antigen.

Investigation of the Properties of the Antibody.

Saline suspensions of an ovine and a bovine strain were heated at 100° C. for two hours and used to immunise two rabbits, the same doses and time intervals as in the immunisation with untreated antigen being observed. The sera obtained were tested for agglutinins for the homologous and heterologous antigens, both heated and unheated, and control tests were carried out, using rabbit serum of the bovine and ovine types against heated and unheated suspensions of both types. Normal rabbit serum failed to cause agglutination of any antigen, the immune sera against unheated suspensions acted as in previous tests, and the sera prepared with heated antigens each caused agglutination of ovine and bovine suspensions to an almost equal but low titre. Heated antigens of the ovine and bovine types thus produce sera which act similarly upon suspensions of both the ovine and bovine bacteria, agglutinating them both to low titre. It may, therefore, be conjectured that the common antigen is a heat-stable one and that the specific antigen is heat-labile. All attempts at causing agglutination of the heated suspension failed. After two days' incubation with the homologous and heterologous sera, prepared with heated and unheated antigens, no trace of agglutination was found. The same result was obtained when the suspension was heated in distilled water and when treatment with alcohol was substituted

for the heating. Re-suspending the bacteria in high concentrations of salt also met with similar results. Moreover, the heated antigen failed to remove agglutinins from its homologous serum prepared with heated antigen.

Discussion.

A study of the antigenic structure of ærobic bacteria, especially of those of the Salmonella group, has revealed the fact that the antigen giving rise to agglutinins comprises a heat-stable " O " antigen and a heat-labile " H " antigen. Robertson and Felix (1928) studied the antigenic structure of the anærobic bacteria *V. septique* and *B. tetani* and established the existence of the same phenomena in them. On analysing the results of the foregoing experiments it is found that the same theory explains all the reactions except the failure of the heated or alcohol treated suspensions to agglutinate. It would seem that the bovine and ovine types possess a common " O " or heat-stable antigen and specific " H " or heat-labile antigens, both types on inoculation producing common stabilotropic and specific labilotropic antigens. The cross agglutination occurs by virtue of the " O " antibody, and the high agglutination titre of the sera when tested against the homologous suspension is due to the " H " antibody. Each antigen can absorb from the homologous serum both " O " and " H " antibodies but can only absorb " O " antibodies from the heterologous strain. Loss of the " O " antibody does not lower the titre of the serum for its " H " antigen. When the antigens are heated both are left with the " O " antigen and the resultant sera therefore possess only " O " antibodies which agglutinate the unheated suspensions of both types to the same low titres.

The bearing of these findings on immunisation is of considerable interest. It has recently been adequately demonstrated by workers dealing with ærobic and anærobic bacteria that the " H " titre of an immune serum bears little relation to its value in protecting animals against infection. Moreover, Robertson and Felix (1930) were able to produce an immune serum against *V. septique* by using suspensions devoid of " H " antigen, and this gave a large measure of protection against injections of actively multiplying bacteria in broth cultures. Hence, if the deduction made that bovine and ovine *B. chauvœi* differ only in their " H " agglutinin is correct, there is good reason to suppose that sera made with the one strain will protect animals against infection with the other but not with the same efficacy as would the homologous immune serum. Indications that this may be correct have been given by subsequent work on immunisation, in that bovine cultures are neutralised when mixed *in vitro* with serum made with ovine strains, and that commercially *B. chauvœi* serum produced with bovine strains protects both guinea-pigs and sheep against lethal doses of cultures of ovine strains, although the immunity is lower than that conferred by a serum made with ovine strains.

Summary.

The antigenic structures of ovine and bovine strains of *B. chauvœi* have been studied by agglutination reactions, absorption of agglutinins, and observation of the effect of heat on the antigen. The results obtained indicate that *B. chauvœi* possesses " O " and " H " agglutinins, that the " O " component is common to strains isolated from sheep and cattle but that the " H " component is specific to each type, ovine and bovine.

Ovine and bovine strains form two distinct " groups " of the bacterial species *B. chauvœi.*

Immune serum derived from bovine strains is likely to confer protection against infection with the ovine type.

Sera.	*Suspensions.*	*Titres of Agglutination.*
Ovine 32	Ovine 47	>1280
„	„ 143	>1280
„	Bovine C, M & W.	160, 160, 40
„ 47	Ovine 32	10240
„	„ 143	>1280
„	Bovine C, M & W.	640, 320, 640
„ 51	Ovine 32	>1280
„	„ 143	>1280
„	Bovine C, M & W.	80, 80, 160
„ 143	Ovine 143	>20480
„	„ 32	>20480
„	Bovine C, M & W.	1280, 1280, 5120
Bovine C	Bovine M	>1280
„	Ovine 32, 47, 51, 143	80, 80, 80, 80
„ M	Bovine C	>1280
„	Ovine 32, 47, 51, 143	80, 160, 160, 80
„ W	Bovine C	5120
„	Ovine 32, 47, 51, 143	320, 160, 160, 640
47 absorbed by 47 antigen	Ovine 47	160
47 absorbed by C antigen	„	5120
C absorbed by C antigen	„	None
C „ „ 47 „	„	„
C „ „ C „	Bovine C	„
C „ „ 47 „	„	2560
47 „ „ 47 „	„	None
47 „ „ C „	„	„
47 prepared with heated antigen	47	160
C „ „ „ „	47	160
47 „ „ „ „	C	320
C „ „ „ „	C	320
All sera	Heated antigens 47 & C	None

With the exception of the bovine strains W, C, and M, all the strains have been isolated from sheep grazing on the Romney Marsh. The ovine strains 32 and 51 are derived from cases of parturient gas gangrene in ewes, 47 from a lamb dead of wound gas gangrene, and 143 from a case of spontaneous gas gangrene in a ewe grazing on the same pasture as the bovine from which strain W was isolated. The strains C and M are derived from cases of bovine blackquarter in other parts of England.

References.

Bosworth, F. J. 1930. Report 11th International Veterinary Congress.

Felix, A. and Robertson, M. 1928. Brit. Journ. Exp. Path, **11**, 14.

Jungherr, E. 1928. J. Inf. Dis., **42**, 85.
Marsh, H., Welsh, H. and Jungherr, E. 1928. Jl. Amer. V.M.A., **27**, 63.
Roberts, R. S. and McEwen, A. D. 1931. Jl. Comp. Path. and Ther. **44**, 180.
Robertson, M. and Felix, A. 1930. Brit. Journ. Exp. Path., **11**, 14.
Viljoen and Scheuber. 1926. Union of S. Africa, 11th and 12th Reports of the Director of Veterinary Education and Research, 499.
Witt and Strickdorn. 1919. Deut. Tier. Woch., **35**, 199.
Zeissler, J. 1924. Deut. Tier. Woch., **32**, 623.
Zeller, H. 1927. Deut. Tier. Woch., **35**, 259.

ARTHRITIS AND PERIOSTITIS IN PHEASANTS CAUSED BY STAPHYLOCOCCUS PYOGENES AUREUS.

By Norman Hole and Harvey S. Purchase.

Ministry of Agriculture Laboratory, Weybridge.

In September, 1929, our attention was drawn to a disease which affected the joints of a large number of young pheasants reared on an estate in North Wales. Our investigations have shown the condition to be a suppurative one due to *Staphylococcus pyogenes aureus*, and as the condition appears to be comparatively rare in birds, and no previous record in pheasants has been discovered, a brief account of symptoms, lesions, and our experimental diagnosis is given in this article.

Kaupp[1] has described an arthritis in geese and ducks due to *S. pyogenes aureus* sometimes associated with intestinal catarrh, whilst Hutyra and Marek[2] state that young geese, young ducks, and chickens may be affected either by *S. pyogenes aureus* or *S. pyogenes albus*. In this respect it is of interest to note that from one pheasant we obtained a pure culture of *S. pyogenes citreus* from the joint lesion and heart blood. Hutyra and Marek describe a peracute septicæmic form of the disease without joint or bone changes, and as a result of our investigations we are of the opinion that the organisms (which gain access to the blood stream through superficial wounds) may either cause a speedy death from septicæmia or become congregated in the joint cavities and set up a chronic arthritis.

Symptoms.

The first symptoms were noted when the birds were between six and ten weeks old; no evidence of infection occurring after the fourteenth week was observed. About 100 birds, or 3 per cent. of those reared, had been lost or destroyed, and this was regarded as very serious in birds that had survived syngamiasis and coccidiosis. The principal symptom was lameness, with a hobbling gait and frequent resting on the hocks. Manual examination usually showed one or more joints to be swollen, particularly the hock (tibio-metatarsal) joints, but sometimes the only visible lesion was a little white corn-like spot under the metatarso-phalangeal joint on the pad of the foot. Birds found dead were sometimes emaciated, but quite frequently were in very good condition. It was noticeable that the more severe the joint lesions the more emaciated was the bird.

Morbid Anatomy and Histology.

In the post-mortem examination of a number of the pheasants lesions were found in connection with the joints in the great majority of cases. In a few birds found dead in very good condition no joint

lesions were found, but the pericardial sac contained several c.c. of clear fluid, and enteritis was occasionally observed; cultural examinations were always positive. One pheasant showed a large ulcerating sore which appeared to originate at the patella joint, with pockets lined with a cheesy membranous deposit extending into the subcutaneous tissue as far as the lumbar region. Both hocks were swollen, and the joint cavities were found to contain cheesy pus, whilst bone erosions were present on the articular surfaces. The lesions in different specimens varied in intensity, pus being sometimes confined to the joint cavity, sometimes extending up and down tendon sheaths or into the bone itself through articular surface erosions; lesions were frequently found in wing joints. Smears were made from the pus in the joint cavities and stained by the methods of Gram and Ziehl-Neelsen; in no case were acid-fast bacilli detected, but in every case Gram-positive staphylococci were seen in enormous numbers. Sections were cut across affected joints, and these showed on microscopical examination abscesses involving the articular cartilages, and the bone itself, and gross changes taking place in the bone marrow. Staphylococci were to be found in all the tissues involved—cartilage, bone, and bone marrow, and also in polymorphonuclear cells in the marrow spaces. No abscesses were found in the marrow, nor signs of osteomyelitis.

The Causative Agent.

Staphylococci are ubiquitous saprophytes, and in order to satisfy ourselves that they were the primary cause of the arthritis, and not secondary invaders, it was necessary to reproduce the disease experimentally. In a number of cases that showed few or no lesions it was easy to cultivate the organism from the heart blood and liver, as well as from the joints, whilst in more chronic cases the staphylococci, although readily recoverable in pure culture from the joints, were not obtainable from the body tissues. From one bird a pure culture of *S. pyogenes citreus* was obtained from the heart blood and joint cavity. This evidence led us to assume that the disease might occur as an acute septicæmic form or a chronic arthritic form, and we soon found by experimental inoculation that a small dose of the staphylococci, given subcutaneously to healthy pheasants six to ten weeks old, caused their death from septicæmia in one to three days.

The experimental reproduction of the chronic form of the disease proved more difficult, and it was not until we were experimenting with some hyperimmune rabbit serum on some older birds that we reproduced the identical chronic lesions in the joints. It would seem that there is an age-immunity to death from the septicæmia, but subcutaneous inoculation in these older birds resulted in the localisation of the infection in the joints. The two birds inoculated were four to five months old, and they died 21 and 28 days respectively after inoculation of the staphylococci. Post-mortem examination revealed swelling and pus in the hock and other joints of both,

and a section made from the joint showed a similar picture under the microscope to that seen in natural cases. We were now satisfied that the staphylococcus was the causative agent.

The Organism.

This grew readily under anærobic or ærobic conditions on agar slants and in peptone broth and produced a growth just like *Staphylococcus pyogenes aureus*. It formed acid in lactose, glucose, mannite, maltose, dextrine and lævulose, and acidified and clotted litmus milk in 24 hours ; indol was not produced and gelatine was liquefied. According to Hine[3] the organism belongs to the pathogenic group of staphylococci since it ferments mannite.

Pathogenesis.

A representative group of the inoculations carried out is included in Table I. Twenty-four hour broth cultures were used for the inoculations, and the organism never failed to kill pheasants less than ten weeks old within four days when given subcutaneously in doses of 0·1 c.c. or more. Frequently no lesions were discoverable, but the organism was always recovered in pure culture from the tissues. Adult fowls and ducks appeared to be refractory, and rabbits resisted infection with small doses given intravenously. Several ducklings were inoculated, and they all recovered very rapidly from a lameness which appeared between the third and fourth days after injection.

Natural Infection.

With the object of discovering the method of natural infection a visit to the estate was made, in company with Dr. Montgomerie, of the University of North Wales.

The birds had three distinct permanent ranging grounds, and it was in pheasants on one of these grounds only that the disease occurred. The disease was not new, but had only assumed serious proportions in 1929. Previous to 1928 no pheasants had been reared on the estate for many years, but the head keeper remembered a similar trouble before the pheasant-rearing was abandoned 20 years ago. No special batch of birds could be incriminated, and the disease never appeared on the ground set aside for rearing, from which the birds were transferred when five weeks old. The same food was fed to birds on all the ranging grounds, so we confined our attention to the infected ground, as it seemed that wound infection was the most probable hypothesis. This particular ground was made up of a stretch of park-land bordered on one side by woods. The striking feature of the ground was an enormous growth of thistles ; they had been cut and were lying in dry heaps in cartloads. We could see that these heaps formed a favourite " squat " for the birds, and this fact was readily confirmed by keepers, who also stated that young birds do not take to the woods at night, but

squat in the open. Thistles were not present in appreciable quantity on the other ranging grounds.

Prevention.

It seemed probable that natural infection occurred through wounds, possibly contracted from repeated scratching of the tender skin by the hard dry prickles. It was suggested that the particular ranging ground should be temporarily abandoned and efforts made to control the thistles, or, if this was not practicable, that the thistles, should be cut early in the year and removed or burned.

Experimentally, a few attempts at protection were made at the laboratory with dead vaccine (Table II), and with hyperimmunised rabbit sera (Table III), but the results obtained were not encouraging ; very little, if any, immunity was obtained against experimental infection, and, in view of the impracticability of giving a vaccine to the pheasants on the estate on a large scale, the work was abandoned.

TABLE I.

PATHOGENICITY OF THE STAPHYLOCOCCUS.

Animal.	*Inoculum and Route.*	*Result.*	*Post-mortem Findings.*
Fowl 1653	1 c.c. broth culture i/v	nil	—
„ 1618	1 c.c. „ s/c	„	—
„ 1639	1. c.c. „ i/m	„	—
„ 1648	5 c.c. „ *per os*	„	—
Pheasant 1767	0·5 c.c. broth culture s/c	Dead 24th hr.	Pericarditis only
„ 1765	0·1 c.c. „ i/v	Dead 36th hr.	Pericarditis and slight enteritis
„ 1768	3 c.c. „ *per os*	nil	—
„ 1766	Control in contact with above	„	—
„ 1871	0·1 c.c. broth culture s/c	Dead 28 days	Both hock joints swollen. Enteritis
„ 1881	0·1 c.c. „ s/c	Dead 21 days	Much pus in hock joints and tendon sheaths
Duck 1059	1 c.c. broth culture i/v	nil	—
Duckling 2011	0·5 c.c. „ s/c	Went lame	—
„ 2099	0·5 c.c. „ s/c	3rd day but	—
„ 1238	0·5 c.c. „ s/c	recovered	—
Rabbit 1904	0·2 c.c., then 1 c.c. broth culture 5 days later i/v	Dead 6th day	No macroscopic lesions
„ 1934	0·5 c.c. broth culture i/v	Dead 24th hr.	„
„ 1949	0·05 c.c. „ i/v	nil	—
„ 1950	0·05 c.c. „ i/v	„	—

Pheasants 1871 and 1881 were between four and five months old when inoculated ; the other pheasants were under ten weeks.

The organism was recovered in pure culture from the heart blood, liver, and joint cavities of pheasants 1767, 1765, and 1871, and from the joint cavities only of pheasant 1881. Cultures made from the heart blood of rabbits 1904 and 1934 gave only a scanty growth.

TABLE II.

ATTEMPTS AT VACCINATION.

Pheasant.	*Vaccine.*	*Test Dose.*	*Live Culture.*	*Result.*
1875	1 c.c. s/c	0·1 c.c. i/v	6 days later	Died
1877	1 c.c. s/c	0·1 c.c. i/v	9 days later	Died
1763	1 c.c. s/c 1 c.c. i/v 8 days later	0·2 c.c. s/c	6 days later	Died
1873	„			Died
1880	„	0·2 c.c. s/c	10 days later	Died

The vaccine used was a 48 hr. broth culture killed by heating for half hour at 65° C.

TABLE III.

MIXED RABBIT SERUM AND LIVE CULTURE INOCULATION.

Pheasant.	*Inoculum.*	*Result.*
1881	1 c.c. serum + 0·1 c.c. broth s/c	Died 21 days. Joint lesions
1757	1 c.c. serum + 0·1 c.c. broth s/c	Died 20 days. Culture positive

Serum prepared from rabbit 1950, commencing with 0·05 c.c. live culture, then 0·5 c.c., 3 c.c., 5 c.c. of dead culture given intravenously at seven day intervals.

The serum and culture were mixed and incubated for one hour at 37° C. prior to inoculation.

SUMMARY.

(1) A disease affecting the joints and bones of young pheasants has been investigated and found to be due to infection with *Staphylococcus pyogenes aureus*. *S. pyogenes citreus* may rarely also assume the rôle of causative agent. A peracute septicæmic form, with no joint lesions, was occasionally met with.

(2) The organisms appeared to be disseminated primarily by the blood stream, the disease resolving itself either into an acute septicæmia or a chronic arthritis. The reasons for this opinion are that birds dying in plump condition of septicæmia showed no lesions, that birds showing joint lesions were poor or emaciated, and that joints of the wings as well as joints of the leg were frequently found affected.

(3) Infection by wounds seemed the most probable route and thistles were suspected as being the wounding agents.

(4) Efforts at immunisation were not successful, but the subject was not thoroughly investigated as protective inoculation did not appear feasible from the practical standpoint.

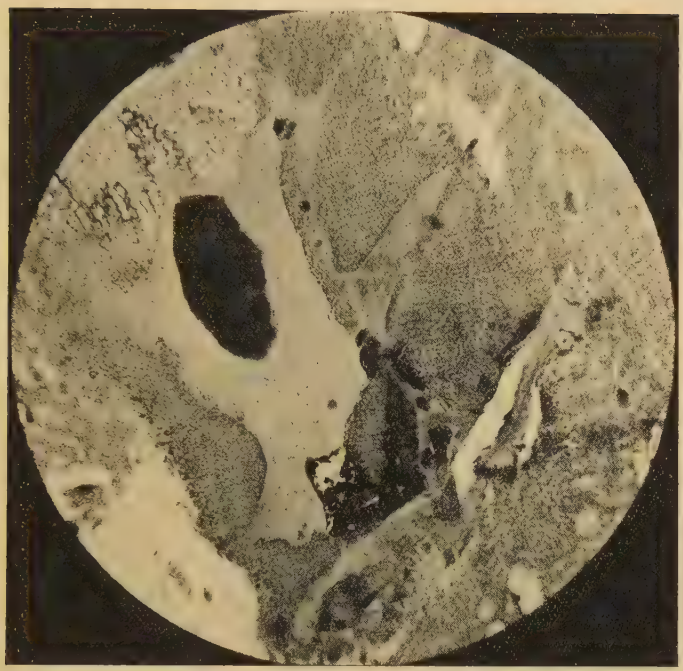

FIG. 1.

Photomicrograph. × 75, Gram's stain. This shows destructive changes taking place in the cartilage of the head of the tibia. The very dark areas are masses of staphylococci. The marrow also shows marked reactive changes.

REFERENCES.

[1]Kaupp. 1922. Poultry Diseases, 185.
[2]Hutyra and Marek. 1926. Spec. Path. & Thera. Dis. Dom. Animals. *1*, 125.
[3]Hine. 1922. Serological classification of Staphylococci. *The Lancet*, *203*, 1380.

B. PALUDIS (McEWEN)—A COMPARISON WITH THE LAMB DYSENTERY BACILLUS.

J. H. Mason, f.r.c.v.s., Helen E. Ross, b.sc.,
and T. Dalling, m.r.c.v.s.

Wellcome Physiological Research Laboratories, Langley Court, Beckenham, Kent.

In 1930, McEwen described an anærobic organism which he named *B. paludis*. He stated that it had many properties in common with *B. welchii*, and the lamb dysentery bacillus but could be distinguished from both. It is this relationship with which we shall mainly deal.

In response to our request Mr. McEwen kindly sent a culture of *B. paludis* C.T. 40 in a tube of meat broth with a layer of vaseline superimposed. The meat was of an intense pink colour. On opening the tube no disagreeable odour was noticed and smears stained by Gram's method showed only Gram-positive organisms like *B. welchii*.

We have examined this strain and compared it with several strains of the lamb dysentery bacillus.

In 1928 we summarised the differences between lamb dysentery bacillus and *B. welchii* as follows :—

	L.D. bacillus.	*B. welchii.*
(A) Toxin	Highly potent	Potent
(B) Antitoxin	Neutralises L.D. and *B. welchii*	Neutralises *B. welchii* but not L.D.
(C) Solid Serum	Liquefies	Does not liquefy
(D) Alkaline Egg	Clots rapidly	Does not clot
(E) Glycerine	Does not ferment	Ferments
(F) Acrolein	Does not form	Forms

Our examination of the culture *B. paludis* has been mainly confined to these six points, though our opinion is that the organism is indistinguishable morphologically from *B. welchii* and the " lamb dysentery bacillus" (referred to in the course of this paper as the L.D. bacillus).

(A) *Toxin.*

In suitable liquid medium (*e.g.*, Robertson's meat broth) *B. paludis* produced an exotoxin as potent as that produced by the L.D. bacillus. The toxin was precipitated by saturation of the toxic filtrate with ammonium sulphate ; we have employed this precipitate dried *in vacuo*. As small an amount as 0·005 mgm. (in solution in saline) when injected intravenously caused the death of a mouse. The toxin is as potent as any we have been able to produce from the lamb dysentery bacillus and many times more potent than that produced by any strain of *B. welchii* with which we have worked.

(B) *Antitoxin.*

We prepared antiserum in two rabbits by injecting them subcutaneously, at bi-weekly intervals, with 14 increasing doses of *B. paludis* toxin (from 0·5 c.c. to 5·0 c.c.). The serum was tested for protective value against the toxins of *B. paludis*, L.D. bacillus, and *B. welchii* by the intravenous injection of mice with mixtures of the serum and the respective toxins.

TABLE I.

Toxin.	*Amount of Toxin.*	*Amount of Antitoxin (Paludis).*	*Result.*
B. paludis	2·0 mgms. (250 fatal doses)	0·12 c.c.	L L L
		0·1 c.c.	L L L L L +
		0·09 c.c.	L +
		0·08 c.c.	+ + +
Lamb dysentery	2·0 mgms. (100 fatal doses)	0·05 c.c.	L L
		0·04 c.c.	L L L
		0·035 c.c.	+ +
		0·03 c.c.	+ +
B. welchii	2·0 mgms. (6-7 fatal doses)	0·2 c.c.	L L L
		0·1 c.c.	L L L
		0·03 c.c.	+ + +
		0·01 c.c.	+ + +

+ = died. L = lived.

Result.

B. paludis antitoxin, therefore, protected against all three toxins.

We next tested lamb dysentery antitoxin and *B. welchii* antitoxin against the three toxins.

TABLE II.

Toxin.	*Amount of Toxin.*	*Amount of Antitoxin (Lamb Dysentery A. 12479).*	*Result.*
B. paludis	2·0 mgms.	0·01 c.c.	L L L L
		0·009 c.c.	L L L
		0·008 c.c.	L L L +
		0·007 c.c.	+ + +
		0·006 c.c.	+ + +
		0·005 c.c.	+ + +
Lamb dysentery	2·0 mgms.	0·003 c.c.	L L L
		0·0025 c.c.	+ + +
B. Welchii	2·0 mgms.	0·012 c.c.	L L
		0·01 c.c.	L +
		0·008 c.c.	+ +

Result.

L.D. antitoxin protects against the three toxins.

TABLE III.

Toxin.	*Amount of Toxin.*	*Amount of Antitoxin (B. welchii G.G. 2748).*	Result.
B. paludis	0·01 mgms. (one sure fatal dose)	0·1 c.c.	+++
Lamb dysentery	0·02 mgms. (One sure fatal dose)	0·1 c.c.	+++
B. welchii	4 mgms. (12-14 m.l.ds.)	0·005 c.c. 0·0045 c.c. 0·004 c.c. 0·0035 c.c.	L L L L L L L + L +++ ++++

Result.

B. welchii antitoxin neutralises *B. welchii* toxin but not the other two toxins.

In addition, one sure m.l.d. of *B. paludis* toxin was titrated against 0·1 c.c. of each of six other *B. welchii* antitoxins, two *B. œdematiens* antitoxins, two *Vibrion septique* antitoxins, one *B. histolyticus* antitoxin and the sera of three normal horses. In no instance was neutralisation of the toxin produced. Similar experiments have been conducted many times with L.D. toxin and again, in no instance, has there been evidence of neutralisation.

Table IV shows that approximately the same result is obtained whether *B. paludis* or L.D. toxins be used in the titration of either *B. paludis* or L.D. antitoxins by the mouse i.v. method. Two samples of *B. paludis* toxins (" A " and " B ") and one L.D. toxin, and two *B. paludis* (rabbit) antitoxins (" A " and " B ") and one L.D. (horse) antitoxin were employed.

TABLE IV.

Toxin.	*m.g.*	*L.D.*	*Neutralising dose of antitoxin.*	
			Paludis " A."	*Paludis " B."*
Paludis " A "	2	0·01 c.c.	0·2 c.c.	——
Paludis " B "	2	0·008 c.c.	——	0·1 c.c.
L.D.	2	0·003 c.c.	0·07 c.c.	0·04 c.c.

Result.

It will be seen that it takes three times (0·01/0·003) as much antitoxin (whether L.D. or Paludis " A ") to neutralise 2 mgm. of Paludis " A " toxin as it does to neutralise L.D. toxin ; similarly,

it takes two and a half times as much antitoxin (whether L.D. or Paludis " B ") to neutralise Paludis " B " toxin as to neutralise L.D. toxin.

Titrations with both toxins carried out intradermically in guinea-pigs gave comparable results.

Active Immunisation.

Anacultures (toxoided whole cultures) were made by the action of formalin on cultures of *B. paludis* and L.D. bacillus. Two groups of guinea-pigs were injected with the respective antigens so produced, two subcutaneous inoculations of 2 c.c. being given at an interval of 14 days. Eleven days after the second injection the guinea-pigs were tested for immunity by the intravenous injection of the toxins of the two organisms ; the results are summarised in Table V.

TABLE V.

Group A.—Guinea-pigs Immunised with *B. paludis* Anaculture.			
G.P.	*Tested with* B. paludis *Toxin.*	*G.P.*	*Tested with L.D. Toxin.*
1	Withstood 4 fatal doses not 8	1	Withstood 16 fatal doses not 32
2	„ 4 „ „ „ 2	2	„ 32 „ „ „ 64
3	„ 4 „ „ „ 8	3	„ 64 „ „ „ 128
4	„ 8 „ „ „ 16	4	„ at least 128 fatal doses
5	„ 8 „ „ „ 16	5	„ „ „ „ „
6	„ 8 „ „ „ 16	6	„ „ „ „ „

Group B. Guinea-pigs Immunised with L.D. Anaculture.			
G.P.	*Tested with* B. paludis *Toxin.*	*G.P.*	*Tested with L.D. Toxin.*
1	Withstood 1 fatal dose not 4	1	Withstood 4 fatal doses not 8
2	„ 1 „ „ „ 4	2	„ 8 „ „ „ 16
3	„ 8 „ „ „ 16	3	„ 8 „ „ „ 16
4	„ 8 „ „ „ 16	4	„ 16 „ „ „ 32
5	„ 8 „ „ „ 16	5	„ 32 „ „ „ 64
6	„ 8 „ „ „ 16	6	„ 32 „ „ „ 64
		7	„ 32 „ „ „ 64
		8	„ 32 „ „ „ 64

(In testing the degree of immunity produced by L.D. antigen, advantage is taken of the fact that the toxin kills with great rapidity when injected intravenously, one certain lethal dose producing death within one hour, and usually after ten to 20 minutes. The routine procedure is as follows :—Doses ranging round a fatal dose, previously accurately determined in a large number of guinea-pigs, are intravenously injected (cardiac puncture) into a number of controls, just prior to testing immunised guinea-pigs to ensure that no mistake in technique (weighing of dry toxin, &c.) has occurred on that day. On the death of the controls all animals under test receive a fatal dose. After one hour survivors receive a further 3 m.l.ds. and to the survivors of this injection a further four lethal doses are given. Later eight, and finally 16 fatal doses are injected, making in all 32 m.l.ds. We have shown on a number of occasions that the result by " cumulative " method agrees closely with that obtained by injecting a large number of fatal doses at one time, guinea-pigs from the same group being employed.)

Table V shows that *B. paludis* and L.D. anacultures cross immunise when tested in guinea-pigs. We have no experiments to show that *B. paludis* anaculture will immunise guinea-pigs against *B. welchii* toxin, but previous work has shown that it is difficult to immunise guinea-pigs against *B. welchii* even when a *B. welchii* antigen is used. We have shown, however, that three to six subcutaneous injections of L.D. anaculture will so immunise that the guinea-pigs withstand one to two fatal doses of *B. welchii* toxin.

It is of interest that both groups of guinea-pigs withstood more m.l.ds. of L.D. toxin than of paludis toxin. With these same batches of toxin the same result has been obtained in three different experiments. We might explain this difference by supposing that the batch of paludis toxin had become more toxoided since its original preparation than the batch of L.D. toxin used. Yet when measured against both of these toxins, the two antitoxins have the same potency. Thus, whether titrated against 1 m.l.d. (0·5 mgm.) of paludis toxin or 1 m.l.d. of L.D. toxin (1·4 mgm.), the particular batch of L.D. antitoxin used was ten times stronger than the paludis antitoxin, for it took 0·002-0·0021 c.c. of L.D. antitoxin to neutralise either toxin, whereas 0·025 of the particular batch of paludis antitoxin was needed.

We investigated further why these immunised guinea-pigs withstood more L.D. toxin than paludis toxin.

(*a*) Seventeen guinea-pigs were immunised by three subcutaneous injections of paludis anaculture, 14 days interval being allowed between the first and second injection, and seven days between the second and third. Nine days after the last injection blood was withdrawn from the heart of each animal and the serum obtained titrated (i.v. mice) against approximately the same antitoxin-binding test dose of both paludis and L.D. toxins. (These doses were equivalent to about ± 20 per cent. and the titrations were carried out at 100 per cent. decreasing levels of antitoxin). On the next day, the animals were divided at random into two groups, and one lot was tested by the intravenous injection of the L.D. toxin and the other with paludis toxin (tolerance test). Table VI shows that titration for the antitoxin content of each guinea-pig (*in vitro* test) gave almost identical values whether L.D. or paludis toxin was used, whereas the tolerance to intravenous injection of the two toxins differed greatly. (Seven out of eight " L.D." guinea-pigs withstood at least 32 m.l.ds., whilst only one paludis guinea-pig withstood more than 16 fatal doses.) Further, guinea-pig 16, whose antitoxin value by *in vitro* test was four to eight units, supported no more toxin by *in vivo* test than did guinea-pig 10 with an antitoxin value of 0·4 to 0·8 units. (Guinea-pig 17 is of interest in that it died after receiving one m.l.d., its serum also having a low antitoxin value.)

These results of the tolerance test again suggest that the paludis toxin contained much more antitoxin-binding toxoid than the L.D. toxin.

TABLE VI.

G.P.	Unitage of G.P. antitoxin when tested by :		Tolerance in m.l.ds. to :	
	L.D. Toxin.	Paludis Toxin.	L.D. Toxin.	Paludis Toxin.
1	0·8—1·6	0·8—1·6	32	—
2	0·4—0·8	0·4—0·8	16—32	—
3	8·0—16·0	2—4	32	—
4	0·8—1·6	0·8—1·6	32	—
5	0·8—1·6	1·6—3·2	32	—
6	16	8—16	32	—
7	0·4—0·8	0·8—1·6	32	—
8	4—8	4—8	32	—
9	0·8—1·6	0·4—0·8	—	4—8
10	0·8—1·6	0·4—0·8	—	8—16
11	0·4—0·8	0·4—0·8	—	8—16
12	No test	—	—	4—8
13	2—4	2—6	—	4—8
14	0·8—1·6	0·8—1·6	—	16—32
15	0·4—0·8	0·4—0·8 ?	—	4—8
16	2—4	4—8	—	4—8
17	0·2	0·2	—	1

(*b*) Four groups of guinea-pigs were injected intravenously with antitoxin as follows :—

Group (1) received L.D. antitoxin sufficient to neutralise 30-35 G.P. m.l.ds. of either toxin. Group (2) received L.D. antitoxin sufficient to neutralise 5 m.l.ds. of either toxin. Groups (3) and (4) were treated as (1) and (2) except that they received paludis antitoxin instead of L.D. Three hours later each group was tested by the " cumulative " injection of one or other toxin. The results may be summarised as follows :—

Groups (1) and (3) tolerated approximately twice as much L.D. as paludis toxin, and Groups (2) and (4) about 1/3-1/2 more L.D. than paludis toxin.

(*c*) It is well known that in actively or passively immunised animals no reactions occur when one or more skin test doses of toxin are injected intradermically, the degree of immunity (*i.e.*, of circulating antitoxin) determining the number of test doses neutralised. Three guinea-pigs received, intravenously, sufficient L.D. antitoxin to neutralise, *in vitro*, 60 to 70 test doses of either toxin and three more the equivalent amount of paludis antitoxin. Six hours later each guinea-pig, along with controls, was injected intradermically with five increasing doses of both toxins. On one side of the body the L.D. toxin injections were made and on the other approximately the same antitoxin-binding doses of paludis were given. In each animal the tolerance to paludis toxin was less by about one-half than that to L.D. toxin.

These results again suggest that the paludis toxin contains more toxoid, but the difference between the paludis and L.D. toxins was not greater than Ehrlich found between two different diphtheria

toxins. Our experiments therefore give no ground for assuming any immunological difference between the two toxins.

Hæmolysin and Anti-hæmolysin.

The filtrate of a toxic culture of *B. paludis* is hæmolytic when tested against washed red cells of the sheep or rabbit. This hæmolysin is neutralised by anti-hæmolysin prepared by immunising a suitable animal with a toxic filtrate. Further, it is neutralised by L.D. and *B. welchii* anti-hæmolysin and its anti-hæmolysin neutralises the hæmolysins of L.D. and *B. welchii*. It would thus appear that the hæmolysins of the three anærobes are serologically identical.

(C) *Solid Serum.*

In the article (1928) it is stated that all strains of lamb dysentery liquefy solid serum and that " sometimes they require incubation under strict anærobic conditions for several days before liquefaction of the medium occurs, while others liquefy the serum in one or two days." Recently we have again examined several strains of the L.D. bacillus and we are still of the above opinion. *B. paludis* C.T. 40 liquefies solid serum when grown under anærobic conditions for about three days. The result agrees with any of our L.D. strains.

(D) *Alkaline Egg.*

On p. 6 of the article (1928) it is stated that " the lamb dysentery bacillus rapidly coagulates alkaline egg. All strains studied possess this character though . . . the rate at which such coagulation takes place varies considerably with the strain." Our examination of the *B. paludis* culture shows that it also coagulates alkaline egg at a slow rate but not more slowly than some of the lamb dysentery strains.

(E) *Glycerine.*

The culture of *B. paludis* C.T. 40 fermented saccharose, galactose, glucose, lactose, *glycerine*, lævulose, maltose, and dextrin, and failed to ferment salicine, mannite, dulcite, and inulin. The typical " stormy fermentation " reaction is produced by its growth in milk. These reactions are identical with those produced by our cultures of L.D. bacillus with the exception of the reaction in glycerine. We have examined many strains of *B. welchii* but with the exception of the L.D. bacillus all have fermented glycerine.

An experiment was conducted as follows :—Five strains of L.D. bacillus, six of *B. welchii*, and the *B. paludis* culture were grown in 2 per cent. glycerine in 10 per cent. peptone water with litmus as the indicator for four days under anærobic conditions (MacIntosh and Filde's jar). All cultures produced gas. None of the L.D. cultures reddened the litmus whilst all the *B. welchii* and the *B. paludis* cultures did so. The *p*H of the cultures, determined colorometrically,

was for the L.D. culture 6·6-6·7 and for the *B. welchii* and the *B. paludis* strains 5·4-5·5. An uninoculated control tube was *p*H=7·8 at the commencement of the experiment and 7·6 at the end.

(F) *Aldehyde and Acrolein.*

Humphreys (1924) showed that *B. welchii* when grown in glycerine broth produced aldehyde and acrolein. Using Humphrey's technique, we have investigated the action of a large number of strains of both the L.D. bacillus and *B. welchii* in glycerine broth, and in addition, the action of *B. paludis* was tested. The result was that no L.D. strain produced aldehyde or acrolein whilst all strains of *B. welchii* and *B. paludis* gave a positive result.

Summary and Conclusions.

(1) *B. paludis* toxin is much more potent than that of *B. welchii.*

(2) *B. paludis* antitoxin neutralises its own toxin, as well as that of *B. welchii.*

(3) *B. paludis* liquefies solid serum and clots alkaline egg.

(4) In these respects and serologically *B. paludis* and L.D. bacillus appear to be identical. The only difference between them is that *B. paludis* (like *B. welchii*) produces acid when grown in glycerine and yields aldehyde and acrolein.

The whole of the work described was carried out with *B. paludis* C.T. 40. McEwen has recently kindly sent us a further four strains of his organism and these will be examined as soon as opportunity permits.

References.

McEwen, A. D. 1930. Jl. Comp. Path. & Ther., xliii, 1.

Dalling, T. 1928. N.V.M.A., Congress Report.

Humphreys, F. B. 1924. Jl. Inf. Dis., xxxv, pp. 282-290.

NEOPLASMS IN ANIMALS.

By Sir John M'Fadyean.

It has been well said that it is easier to write a book about some subjects than to define them. Neoplasms appears to be one of these. "Autonomous or independent new growths," suggested by Thoma (1894), is probably as satisfactory a definition as any that could be constructed with the same number of words.

The word tumour in its wider sense may be applied to any bulky, solid new growth which is obviously foreign to the place in which it is found, and in that sense neoplasms are usually tumours; but

Fig. 1.
Carcinoma involving the eyelids, eye-ball, and cheek. Probably originated in the conjunctiva.

so also are some inflammatory new formations, as, for example, those that occur in tuberculosis and actinomycosis, which are universally admitted to be caused by visible bacteria. It was long ago recognised that these should not be grouped with neoplasms, and the not very appropriate term "granuloma" (suggested by Virchow) is now generally applied to them. Throughout this article, however, the word tumour is used as a convenient synonym for neoplasm.

The two outstanding characteristics of neoplasms are (1) their apparently spontaneous origin and (2) what has been called their parasitic habit.

The words " apparently spontaneous " should not be construed as meaning more than that the cause of the new growth has not yet been discovered or proved, but they serve to distinguish the neoplasm from inflammatory new growths. The words " parasitic habit " connote the fact that neoplasms generally appear to be as independent of the animal in which they are found as is an actual living parasite of its host. The neoplasms, like the parasites, depend upon their host for nourishment only. Their independence in other respects is also evidenced by the fact that they often continue to flourish while the animal itself steadily loses weight.

The cells of which neoplasms are composed also show independence of the morphological laws that determine the histology of the normal organs and tissues, and, figuratively speaking, they stand in relation to the commonwealth of the body like rebels.

FIG. 2.
Carcinoma of the throat of a dog, involving the lymphatic glands by metastasis.

This independence of the genuine neoplasms is well shown in cases of metastasis, in which the secondary growths, of an epithelial tumour for instance, may grow in places normally without epithelium, such as the lymphatic glands and the bones.

An alleged character of neoplasms that would, if confirmed, establish a fundamental difference between them and the normal cells of the body is that they are not liable to senescence. This was at one time inferred from the fact that the essential cells of certain neoplasms (especially those of the carcinomata), developing in old subjects at the time when the natural powers of cell proliferation are at a low ebb, often exhibit over long periods a remarkably increased rate of multiplication, brought to an end only by the death of the patient. Against this view, however, it might be pointed out that,

once a neoplasm has been started, what may be called the environment of the tumour cells is not identical with that of the parent normal cells, and also that the successful artificial cultivation through many generations of normal cells detached from the body has done much to weaken the belief that tumour cells have a capacity for indefinite multiplication that is denied to normal cells.

Clinical Characters of Neoplasms.

With regard to their local and general effect on the patient, neoplasms fall rather sharply into two classes—the malignant, and the benign or innocent.

The characters that stamp a tumour with malignancy are rapid destructive growth, and proneness to metastasis. By metastasis is meant the development of secondary growths apart from the primary

Fig. 3.
Spleen from the same case as Fig. 2, showing metastatic tumours.

one but derived from it. Such secondary growths may be very numerous and widely disseminated throughout the body. They repeat more or less exactly the histology of the primary growth and are themselves malignant. They mark the places where cells that have escaped from the primary growth have been arrested in blood or lymph vessesls. The most malignant of the neoplasms are the carcinomata, the adenomata, and the sarcomata.

The destructive effect of the malignant tumours is attributable to their infiltrative or invasive method of growth and extension, and the absence of a capsule. The two things are connected, but both are mainly due to the rapid multiplication of the tumour cells. In consequence of the method of extension the actual boundary of a malignant tumour is usually wider than the apparent one.

In the benign tumours growth is usually less rapid, a capsule is generally developed, and metastasis does not occur. The mode of growth is expansive (like a bulbous root in the soil), as opposed to the penetrative method in the malignant growths.

Even the most benign of tumours may have serious and even fatal results by the pressure which they exert on the surrounding tissue, with consequent atrophy and, it may be, interference with the functions of important organs.

A state of cachexia is frequently observed in cases of cancer in the human subject, the patient becoming emaciated and anæmic, while the skin takes on a sallow unhealthy colour. To explain this cachectic condition in man it has sometimes been assumed that metabolic substances of an injurious nature may be formed by

FIG. 4.

Carcinoma of the tongue of a cat. The section is from the margin of the tumour. To the extreme right of the centre transverse sections of muscular fibres are seen surrounding a group of epithelial cells. To the extreme left of the figure the muscular tissue has disappeared, and its place is taken by groups of epithelial cells with a fine rather cellular stroma.

tumour cells and pass into the general circulation, but *a priori* there is no reason to expect that anything foreign to the normal body is generated by tumour cells, and cachexia when it occurs can generally be explained by the destructive effect which the tumours have had on important organs, possibly involving ulceration, loss of blood, and invasion by bacteria.

A point of interest in connection with the ætiology of neoplasmatic disease is that the course is usually non-febrile.

The Incidence of Neoplasms in Animals.

Most extensive statistics provided by hospitals, and official national returns purporting to give the different causes of death in the population of all civilised countries, enable one to form at least an approximately accurate estimate of the frequency of neoplasmatic disease in the human species. That is not possible with regard to the occurrence of tumours in the lower animals (1) because the registration of the death of these is not compulsory except in the case of certain contagious diseases, and (2) because a proper post-mortem examination is made in only an insignificant proportion of the animals that die naturally. Slaughter-house statistics are of little use because as a rule only apparently healthy animals are sent there. The age incidence of some tumours, notably cancers, cannot be estimated in some of the species because the majority of the animals are killed while still young.

Notwithstanding these difficulties, it is possible to say that, with one exception, all the neoplasms that are at all common in the human subject also occur in the domesticated mammals. The exception is the chorion-epithelioma, or chorioma—a malignant tumour of the pregnant uterus; its cells are derived from the epithelium of the villi of the fœtal chorion, which normally penetrate the substance of the uterine wall in the formation of the placenta in women. In this case therefore the cells of which the tumour is composed are direct descendants of the fœtus and not of the mother, in whom the tumour continues to grow and exhibit its autonomy or independence of both the individuals.

The Ætiology of Neoplasms.

To deal with this part of the subject, except in outline, would be beyond the intended scope of the present article.

The two theories that hold the field at the present time are (1) the microbial, and (2) the irritational.

The first of these would bring neoplasms into line with the circumscribed new growths (or tumours in the wider sense of the word) which are admittedly caused by micro-parasites, especially bacteria; the second would attach most importance to prolonged irritation and injury of normal cells.

It should be noted, however, that while the microbial theory is presented as an explanation of the actual origin of neoplasm cells and of their special growth characters, the second theory suggests only that under chronic irritation some of the normal cells acquire and transmit to their progeny the new powers that underlie their ability to build up masses of new tissue even in the absence of demonstrable micro-parasites and in sites where they are no longer exposed to the irritation.

Microbial Theory.

In order that the cogency of the arguments for and against this theory may be appreciated, some facts generally admitted must be mentioned here.

The first is, that the presence of demonstrable bacteria or other micro-parasites in neoplasms is exceptional save in circumstances that make it reasonable to regard their presence as accidental. In fact, it may be asserted that, except when there has been an opportunity for invasion by bacteria as a result of superficial necrosis or ulceration, the tissue of a neoplasm is generally as free from micro-parasites as the surrounding normal tissue. No demonstrable bacterium or other micro-parasite has been proved capable of causing any of the genuine neoplasms.

A remarkable impetus was given to the study of neoplasms by the researches of Jensen, who was able to transmit epithelial tumours normally occurring in mice to animals of the same species by the method known as transplantation or grafting (1903). The method consists in inserting under the skin, with aseptic precautions, a minute fragment of the tumour which it is desired to transmit. A great body of valuable knowledge has been gained in this way, but the scope of such studies has fallen below expectation because it has been found that transmission succeeds only among animals of the same species, and is even uncertain between animals of different breeds. What is still more to be regretted is that the transmission of tumours arising in the larger domesticated animals has generally proved impossible.

A fact of importance discovered through transplanting experiments is that during the growth of a graft the mouse or other animal acquires a certain degree of immunity manifested by resistance to a subsequent attempt to determine the formation of a tumour by the same method with a graft from a neoplasm of the same strain. The great majority of such experiments have been with epithelial neoplasms, but sarcomatous tumours of mice and rats have been transmitted in the same way. In mice a temporary immunity against successful transplantation can also be conferred by previous injections of mouse embryonic skin or even blood.

The successful transmission of malignant neoplasms has been claimed as evidence in support of the microbial theory of tumour formation, but it is evident that such experiments contribute nothing in support of that theory except on the assumption that the tumours from which the grafts were taken contained micro-parasites, which is the actual question in dispute.

In connection with the successful transmission of certain neoplasms by grafts it should be noted that, at least in the higher animals, a neoplasm cannot be originated in that way by grafts composed of normal tissue—a further fact showing the fundamental difference between the essential cells of neoplasms and those of the normal body.

The discovery by Rous that certain growths occurring naturally in the common fowl, and histologically identified as sarcomas, are transmissible by grafting, requires special notice, especially because transmission of these can also be effected by apparently cell-free

filtrates made from the growth. It may be said, however, that even if it should be established beyond any doubt that some tumours can be transmitted without the introduction of tumour cells it would be rash to accept the fact as an explanation of the origin of neoplasms in general.

There may also be mentioned here the so-called " infective sarcoma " of the dog, which some have accepted as a genuine neoplasm that is experimentally transmissible. This is not warranted, since the growth deserves, meanwhile at least, to be kept apart, because, unlike the genuine neoplasms of the mammalia, the disease is undoubtedly contagious, and apparently is transmitted exclusively by the act of coition in natural circumstances. It has also been shown by Bashford and others that when fragments of this infective sarcoma are transplanted the resulting growth is not produced by multiplication of the transplanted cells. These perish, and the new growth is composed of cells derived from the connective tissue of the experimental dog. The fact that other species than the dog can be infected with this disease by inoculation, and that recovery sometimes occurs both in naturally and experimentally infected animals, indicates that one is here dealing with something that is fundamentally different from the true neoplasms.

An insuperable difficulty in the way of accepting bacterial infection as the common cause of neoplasms is presented by the fact already mentioned, that transmission by transplantation is impossible except between animals of the same species. Acceptance of the theory would therefore involve the belief that to estimate the number of species of bacteria concerned in the causation of neoplasms one would have to multiply the number of animal species susceptible to the infection by the number of histologically different neoplasms. This would give a fantastic number of different tumour-producing micro-organisms even if the question were confined to the causation of the malignant neoplasms. It would also have to be assumed that these organisms are ubiquitous, since all the cancers and other tumours occur throughout the world and experience contradicts the view that the disease is spread by contagion. The alleged occurrence of endemic cancer and " cancer houses " has not withstood critical investigation.

Irritation and Injury.

Certain neoplasms of man have long been regarded as the result of continued irritation of the place at which the tumour arose, such as cancer of the lip in smokers, cancer of the scrotum in chimney sweeps and mule-spinners ; and in quite modern times the frequency of cancer of the skin in persons following particular occupations has forced itself on the notice of the medical profession and even of the public. Conspicuous examples are the occurrence of cancer in workers in connection with tar, paraffin, and other mineral oils. By the intentional application of such substances to the skin of mice one can readily cause the development of a malignant carcinoma with the

essential characters and histology of tumours of that class arising in connection with the skin in man.

Such carcinogenic substances do not act with equal effect on all animals ; for example, the production of cancer in that way is more difficult in rabbits than in mice. In experiments of this kind the earliest histological changes are such as might be expected from the repeated application of an irritant substance to the skin, inducing excessive and irregular multiplication of the epithelial cells and irregular overgrowth of the underlying dermis. The normal papillæ of the dermis become overgrown and distorted, with the result that the surface assumes a warty appearance. This has been termed a " pre-cancerous " condition, but without implying that it is always followed by the development of an actual carcinoma. The microscopical evidence that the cancerous process has actually begun in such cases is obliteration of the normally sharp line of demarcation between the epithelium and the *cutis vera* and the penetration of the latter by groups of epithelial cells. The continued growth of the epithelium into the connective tissue implies that its cells have acquired the independence which is the essential character of neoplasm cells.

Such stages are, of course, seldom or never observable in tumours arising otherwise than by experiment, but there is no reason to doubt that the process is identical in tumours arising naturally.

A fact that needs to be emphasised is the chronic or sustained nature of the injury or irritation which is most likely to bring about the change from the normal into cancer cells, especially when the injurious agent is a chemical one.

Interesting and important as the experimental production of cancer of the skin has been, the results do not appear to be immediately applicable in explanation of the origin of neoplasms in general. Such concentrated and sustained chemical irritation can hardly be imagined in connection with the deeper tissues and organs, such as the spleen, thyroid gland, pancreas, and kidney, in all of which tumours often develop.

It has also to be recognised that, even in the case of some of the substances proved to be carcinogenic—shale oil, for example—the proportion of the total workers exposed to the irritation who develop cancer is small. The fact suggests that individual predisposition may be an important factor in the genesis of neoplasms.

Bruises and other forms of mechanical injury have sometimes been accused as the cause of particular neoplasms, but general experience of the occurrence of the disease in human beings and animals indicates that such a connection is rare.

Repeated injury of the root of the horn by the yoke has been considered the cause of cancer of the horn-core in draught oxen in India, where the condition is apparently common although it is rare in Europe.

Burns of the skin in man have been included among the causes of cancer, and tumours of that nature have been found in cattle at

the site of branding with a hot iron. But the firing and blistering of the legs of countless thousands of horses does not appear to have excited the formation of cancers in that position.

In making a post-mortem examination of three wild brown rats (*Mus decumanus*) Fibiger (1913) found in each of them disease of the stomach which microscopic examination showed to have the characters of a carcinoma. It was subsequently found that there was an actual enzootic of this disease in the district. By careful experiments he showed that the disease, which also affected the œsophagus, was caused by a previously undescribed nematode belonging to the genus Spiroptera, which, in the fully developed condition infested the squamous epithelium of the anterior part of the stomach. The intermediary host of the worm was found to be a cockroach, and by feeding rats with cockroaches Fibiger was able to infect them (both wild rats and tame laboratory rats) with the disease. The earliest stage of the disease took the form of an inflamatory overgrowth of the epithelium, followed later by a papillomatous growth which sometimes eventually occupied the whole cavity of the stomach. This papillomatous condition was followed by the development of malignant epithelioma with heterotopic growth of the epithelium, and metastasis in two or three cases. No nematodes or eggs were found in the secondary tumours—a fact indicating that these had to be ascribed to the power of the epithelial cells of the stomach to develop in foreign organs without the help of a parasite. Fibiger felt justified in assuming that the whole of the anatomical changes were caused by the irritative effect of a poison produced by the nematodes.

It was found later that several different species of cockroach could serve as intermediary hosts of the Nematode.

The most common of chronic inflammatory processes in the domesticated animals are those caused by bacteria, such as tuberculosis, glanders, actinomycosis, and botryomycosis, but such lesions appear to play a negligible part in the causation of malignant neoplasms.

Immense numbers of sheep in all parts of the world have their bronchi and air-cells heavily infested with nematode worms and their eggs and embryos, often for many months at a time, but it has not been noticed that cancer of the lung is at all common in that species. The same may be said about the verminous infestation of the true stomach in these ruminants and of the intestines in the horse.

The larvæ of the *Gastrophilus equi*, which are very common parasites in the stomach of the horse and attach themselves firmly to the mucous membrane for nearly a year, have not been recognised as a cause of cancer notwithstanding the fact that the irritation is severe and excites changes that closely resemble the " pre-cancerous condition."

Other Theories.

The theory associated with the name of Cohnheim is one of the oldest. It attributes the origin of neoplasms to the belated activity of groups of cells of the embryo which, it is assumed, failed to share in the normal multiplication and differentiation that bring into existence the various tissues and organs of the fœtus. It is held that such groups of cells (or embryonic " rests ") may have failed to achieve their normal development at the proper time, either from redundancy or accidental detachment.

The theory was for long considered the most satisfying explanation of certain heterotopic tumours, developing in unexpected places, such as cartilage in the parotid gland in man and in the mammary glands in the bitch. In fact, however, primary growths exhibiting the features characteristic of true neoplasms are rarely heterotopic in position or so placed as to compel the belief that they cannot have had their starting point in the fully differentiated tissue which they resemble histologically.

It may be admitted that the theory offers a plausible explanation of the origin of certain growths with a complex structure which are already present before birth in hetorologous positions, and which are now called teratomas to distinguish them from the neoplasms. (*See* p. 284) But it is practically without value for the understanding of the great problem of tumour pathology—the ætiology of the malignant neoplasms.

Gye (1925), working with a strain of Rous's No. 1 chicken sarcoma, claimed to have cultivated a virus from it. He found, however, that the virus alone was unable to bring about the malignant transformation of a cell. An adjuvant was necessary and was found in abundance in the substance of the chicken sarcoma ; this adjuvant he called the " specific factor."

As a result of his researches he had been led " to look upon cancer —using the term in its widest sense—as a specific disease caused by a virus or group of viruses. Under experimental conditions the virus alone is ineffective ; a second specific factor obtained from tumour extracts ruptures the cell defences and enables the virus to infect. Under natural conditions ' continued irritation ' of tissues sets up a state under which infection can occur. . . . The virus probably lives and multiplies in the cell and provokes the cell to continued multiplication ! "*

In concluding this part of the subject, it may be said that the weight of the available evidence supports the view that the tumours which are only transmissible by transplantation are not caused by bacteria, and that those transmissible by cell-free filtrates are probably inflammatory growths caused by ultra-visible viruses and should

**The Lancet*, July 18th, 1925, page 117.

therefore not be included in the same category as the former, to which alone the term neoplasm should be applied.

If this view be accepted, it must be admitted that the actual cause of neoplasms has not yet been determined, although it would appear that sustained irritation by chemical and other agents is a factor in the causation of the carcinomata.

Heredity.

Even with regard to neoplasmatic diseases in man, it is very difficult to assess the importance of special susceptibility transmitted by heredity, and it is still more difficult to obtain the evidence that would be necessary regarding the disease in animals. Experiments with mice have shown varying individual resistance to the transplantation of cancerous tumours, and it is quite possible that the readiness with which the larger animals react to the agencies that cause cancer may vary to some extent; and, like other natural characters, any such special susceptibility or resistance would tend to be transmitted to the offspring. If, however, such an influence is at work among farm animals it has hitherto escaped notice.

The Epithelial Neoplasms.

The three members of this group are the carcinomata, the adenomata, and the papillomata.

The Carcinomata.

The carcinomata may arise in connection with the normal epithelium of any part of the body—the skin and mucous membranes and the epithelium of the secreting glands and their ducts.

All these tumours contain what is generally termed stroma as well as epithelium, but the latter is the only essential element, a fact that was first proved by microscopic study of the fate of grafts. That showed that the connective tissue part, or stroma, of the graft suffers absorption and that only its epithelial cells survive to produce the new tumour. As the latter grows, a supporting stroma is provided by activity of the connective tissue cells of the host, and it is therefore to be regarded as a reaction product, not sharing the independence which belongs to the epithelial cells. New vessels are formed in a similar way, and are confined to the stroma.

The proportion of stroma varies greatly in different cases, and its amount largely determines the consistency of the growths.

In the very firm variety—so-called scirrhous cancer—the stroma may greatly exceed the epithelium in amount.

The form of the epithelial cells depends to a large extent on that of the parent epithelium from which the tumour took its origin. Varieties known as squamous, sphæroidal, and columnar are thus explainable. Cancers arising from the stratified squamous epithelium of the skin are known as epitheliomata; they disclose their descent

by the flattened form of the cells and the horny transformation which many of the older ones have undergone. Towards the centre of the larger masses of epithelium, the completely horny cells are often arranged concentrically to form " pearl nests." (See Fig. 6). In these cases the central cells of the group are the oldest because multiplication has been most active at the periphery, where the cells are in contact with the vascular stroma and have the best supply of nourishment Cornification and pearl nests may be found in unexpected situations, such as cancer of the mamma and uterus, in which the normal epithelium is not stratified or liable to the horny transformation.

What is distinctive of the epithelium in the carcinomata is its disorderly arrangement, without the formation of tubes or acini, or basement membrane. When very luxuriant, it is liable to mucoid or other degeneration or actual necrosis.

The carcinomata are always malignant. Metastasis usually occurs at a very early stage, and transport of the cells is mainly by the lymphatic vessels. Blood transport is rare, no doubt because the newly-formed vessels are confined to the stroma and have normal walls.

Surgical removal of the primary tumour is not likely to be successful if it is impossible to include the nearest group of lymphatic glands in the operation.

The epitheliomata are the least malignant, and the primary growth may remain small while the metastatic growths have attained to a large size.

Correct diagnosis demands microscopic examination of a stained section, without which no tumour should be recorded as a carcinoma.

For reasons already mentioned, it is impossible to say whether neoplasms as a whole are more frequent in man than in any of the farm animals, but there certainly appears to be a striking disparity in the case of the tongue, the uterus and the mammary glands. In the latter position, such tumours appear to be more frequent in the bitch than in the other animals, but probably many of the recorded cases were incorrectly diagnosed.*

The rarity of carcinoma of the cow's udder is the more remarkable because of the great frequency of chronic mastitis in that species.

As in man, animals developing carcinomata are usually approaching middle age or older.

Figs. 13 and 14 are of interest in connection with the diagnosis of tumours *intra vitam*. They are from part of what appeared to be a malignant growth in connection with the upper jaw of a filly. The growth caused a swelling on one side of the face, and in the mouth there was a large amount of new soft tissue with an offensive odour. Part of the tumour was received for examination in 1926, before the animal had been seen, and when sectioned it showed the structure

* In 1890 (in this Journal, p. 154) the author called attention to the fact that in the bitch cases of chronic mastitis with enlargement and induration were often mistaken for cancer by veterinary surgeons. Not rarely several glands are affected to a like degree and, of course, without metastasis.

seen in the two micro-photographs. In consequence, it was reported that the growth was a carcinoma, and it was then learned that the animal was a filly under two years old. The animal was afterwards admitted to the College Infirmary, and, in view of its age, the previous diagnosis was withdrawn. A radical operation was out of the question, but as much as possible was removed from the growth in the mouth (by my colleague, Professor Wooldridge). At that time the submaxillary lymphatic glands on the same side as the tumour were tumefied, but not nodular.

The swelling gradually diminished in size, and the filly was returned to its owner. It has recently been learned that it is alive and well, with only a slight deformity at the site of the tumour.

The Adenomata.

The adenomata have their origin in secreting glands, which is a source common to them and the carcinomata, but, unlike the latter, they are seldom or never derived from the general epithelial covering of the skin and mucous membranes. They may arise in connection with any of the large secreting glands and also from the smaller glands in the skin and mucous membranes. They agree with the carcinomata in having a stroma as well as epithelium, and here again the latter is the essential element.

Two types may be distinguished, the one having the epithelium arranged in the form of a lining to tubes or acini, and the other being composed of solid groups or strands of epithelium. The first, which is much the commoner, simulates the structure of a mature normal secreting gland, and the latter the early stage in the development of such glands. In both types, the tumours are distinguishable from the carcinomata by the orderly arrangement of the epithelium, and sometimes by a distinct basement membrane between the epithelium and the connective tissue stroma.

The consistency and appearance on section vary nearly as much as in normal glands. Usually they are soft or moderately firm growths with a whitish or greyish tint, and on close inspection of the cut surface with a lens, or even with the naked eye, it may be possible to detect a glandular appearance and sometimes indications of lobulation.

As a rule, the adenomata of macroscopic size are easily distinguished from the surrounding tissue with the naked eye, and they tend to assume a rounded form and may have a capsule.

The glandular tissue of an adenoma is without ducts or outlet and is usually inactive, but sometimes liquid may transude or be secreted into the acini with consequent formation of cysts. From the wall of such cysts villus-like simple or branched processes may project into the interior. A tumour showing this type of structure is termed a papillary cystadenoma.

A comparatively common adenoma with an interesting structure

FIG. 5.

The section here shewn was taken from the margin of a carcinoma of the fauces of a horse. It shows the appearance which is known as pre-cancerous. The epithelium on the right is nearly normal, but on the left there is a gradually increasing irregularity, with over- growth of epithelium and apparent penetration of the connective tissue by the downward growth of the epithelium.

FIG. 6.

Epithelioma of the penis of a horse. It shows the irregular groups of squamous epithelial cells and the abundant fibrous stroma. Pearl nests are seen at the centre of the figure in the largest collection of epithelial cells. × 108. (The micro-photograph of which the figure is a reproduction was made from a picro-carmine-stained section which had been mounted in Farrants' medium 20 years previously.)

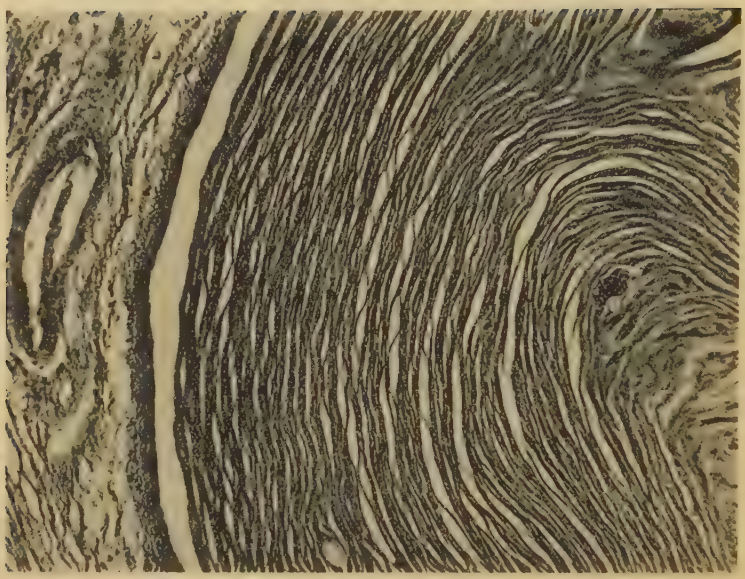

FIG. 7.
From a case of carcinoma of the tongue of a horse. About half of a pearl nest which formed the centre of the mass of epithelium is seen below the centre at the right edge. This is surrounded by a remarkable whorl of elongated strap-like fibres. A higher magnification than was used for the present figure showed nuclei at intervals in these fibres, indicating that they had been formed by fusion of epithelial cells. × 108.

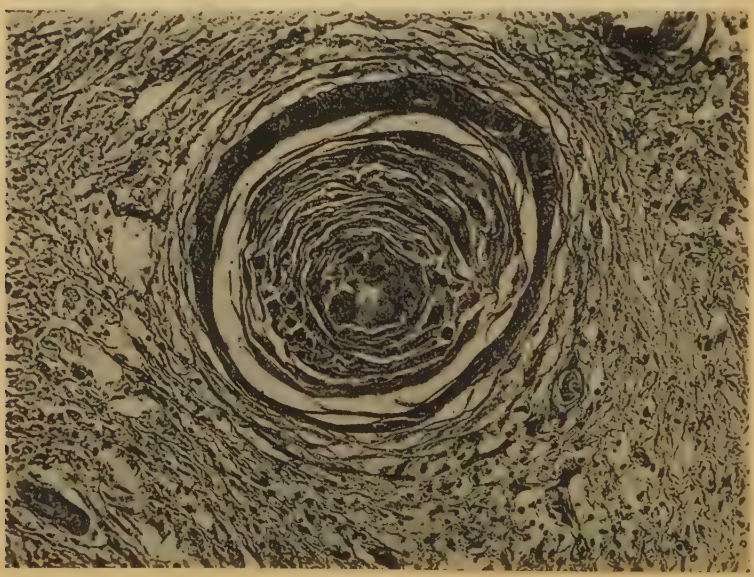

FIG. 8.
From the same case as Fig. 7. × 150.

FIG. 9.
From a case of carcinoma of the eyelid of a mare. Persisting remains of gland structure are seen to the left of the centre. × 67.

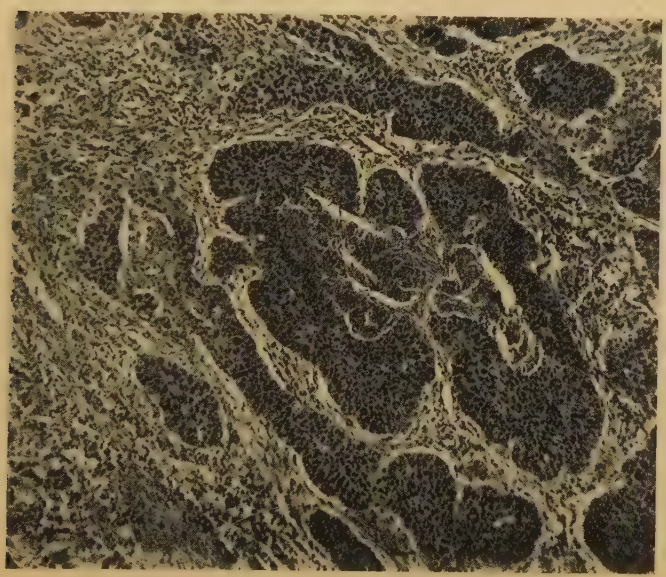

FIG. 10.
From a carcinoma of the orbit of a horse. The central cells of the largest group of epithelium have undergone necrosis. The epithelium shows no keratinisation or pearl nests. Probably originated in the conjunctival epithelium. × 108.

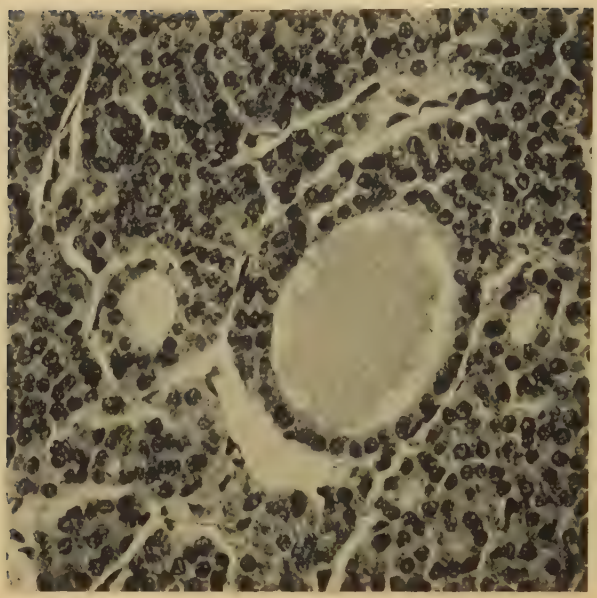

FIG. 11.
Adeno-carcinoma from the neck of a dog; probably originated in the thyroid gland. × 400.

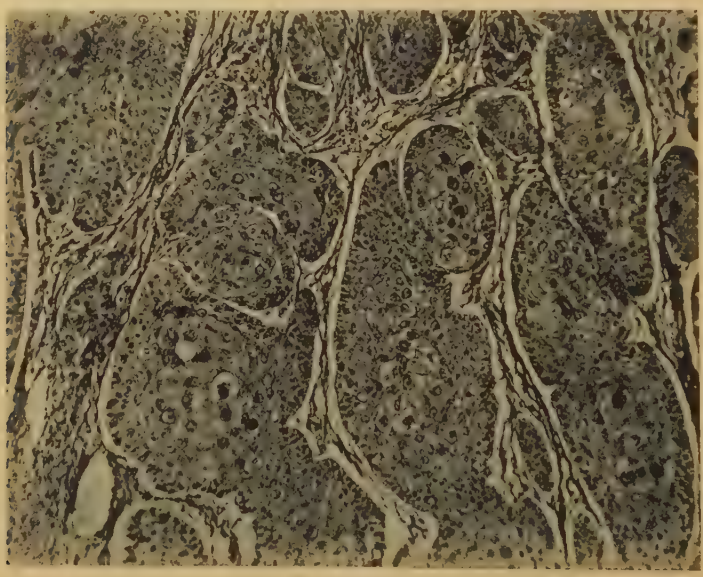

FIG. 12.
From a carcinoma of the upper jaw of a horse. The head was not available for examination, but the tumour probably had its origin in the superior maxillary sinus. The stroma is scanty and the central cells in the epithelial masses are degenerating. × 108.

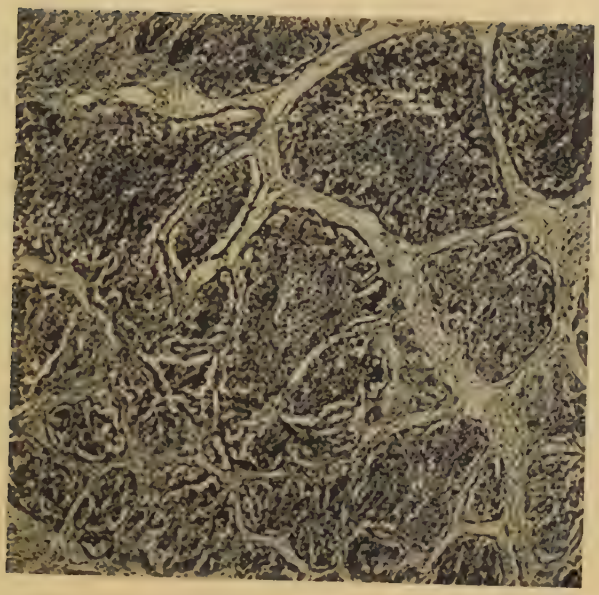

FIG. 13.
From a tumour simulating a carcinoma (*see* p. 277). × 50.

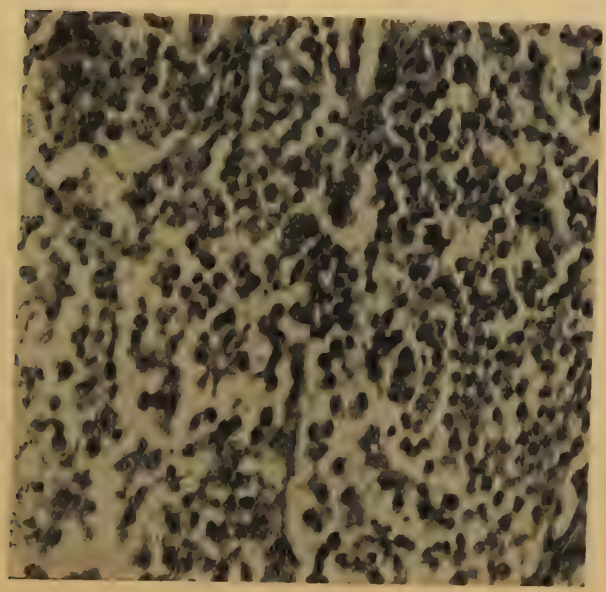

FIG. 14.
Part of Fig. 13. × 170.

is the one that occurs in relation to the anus of the dog, and is well-known to veterinary surgeons as the " anal adenoma " of that animal. It appears to arise from cells of the circum-anal glands of the region. It represents the solid type of adenoma without an actual lumen in the tubes or acini, and with a distinct cellular basement membrane. (See Fig. 16.) These tumours, which may reach the size of a pullet's egg, are encapsuled, benign, and non-recurrent after removal.

Many of the adenomata appear to be malignant *ab initio*, and others become in part or wholly carcinomatous in structure. Fig. 15 is from a large tumour which grew from the mucous membrane of the bladder of a mare. It also shows the solid type of adenoma, but in sections from another part of the tumour the epithelium had the disorderly arrangement characteristic of the carcinomata.

Hypernephromata.

In human pathology this term covers tumours of the kidney with a varying histology, though all of them are mainly composed of large columnar or polygonal cells with glycogen or myelin droplets in their cytoplasm. In some cases the cells have a real or fancied resemblance to those of the *zona fasciculata* of the adrenal cortex, and they may occur either in the form of solid strings or hollow acini. The tumours have a yellow colour, which is often obscured by blood extravasations, to which they are very liable.

These renal hypernephromata were long supposed to provide the most convincing evidence of the development of neoplasms from embryonic rests (Cohnheim's theory), first because of the resemblance of their cells to those of the *zona fasciculata* of the adrenals, and secondly because groups of cells identified as adrenal rests are sometimes found on the surface of otherwise normal kidneys.

In recent years this view of the origin of the renal hypernephromata has been strongly contested by Nicholson* and others, who hold that such tumours are malformations of the embryonic kidney. Whether that view is correct for all the hypernephromata or not, it seems to be the one best fitted to explain the origin of the two tumours of which Figs. 19 and 20 are photographs. (see p. 287.)

The adrenal theory would be irrelevant to explain the origin of these two growths since there is nothing in their histology to suggest it. What stands in need of explanation is the fact that the tumours contain elements that must have been derived from those embryonic cells from which the most important part of the normal kidney is built up, namely, convoluted tubes. The fact that many of the tubes are engaged in forming Bowman's capsules places their nature beyond doubt, and in sections from Fig. 19 rudimentary glomeruli with blood in them were found.

* Guy's Hospital Reports, Vol. 73, Series 4, pp. 164-193, and *Journal of Path. and Bact.*, Vol. xxxiv, 1931, pp. 771-728.

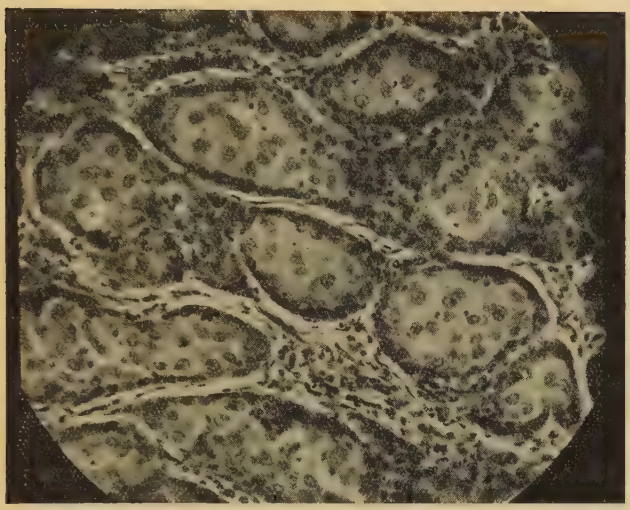

FIG. 15.
Adenoma from the bladder of a mare. It shows the solid groups of epithelial cells without lumen. Stroma is scanty and regular. × 200

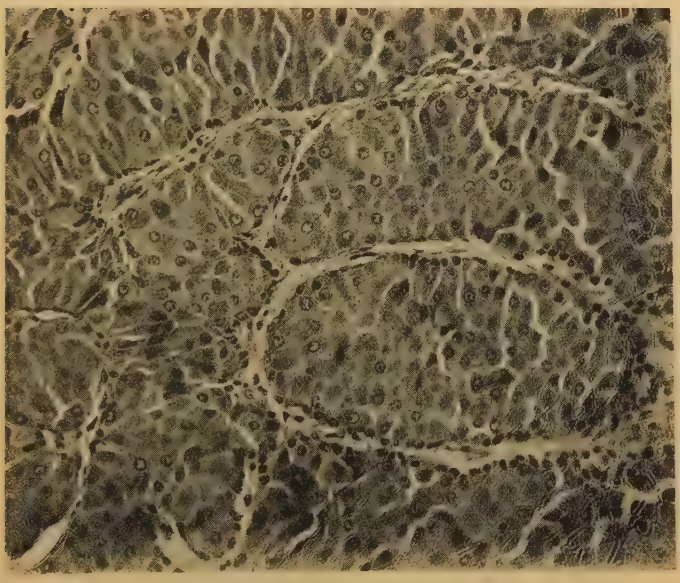

FIG. 16.
Anal adenoma of a dog. It shows the large groups of squamous cells without any lumen, and limited outwardly by a basement membrane with small deeply stained nuclei. × 200.

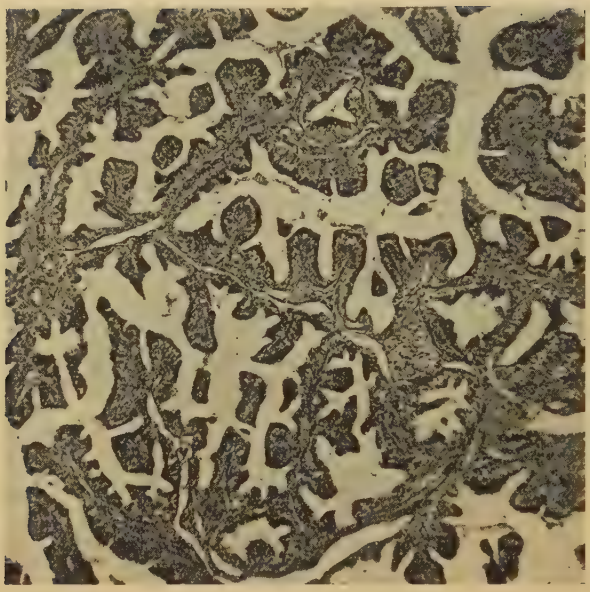

FIG. 17.
Papillary cyst-adenoma of the lung. The lungs contained numerous such tumours metastatic from the liver. Probably arose in connection with the bile ducts. × 50.

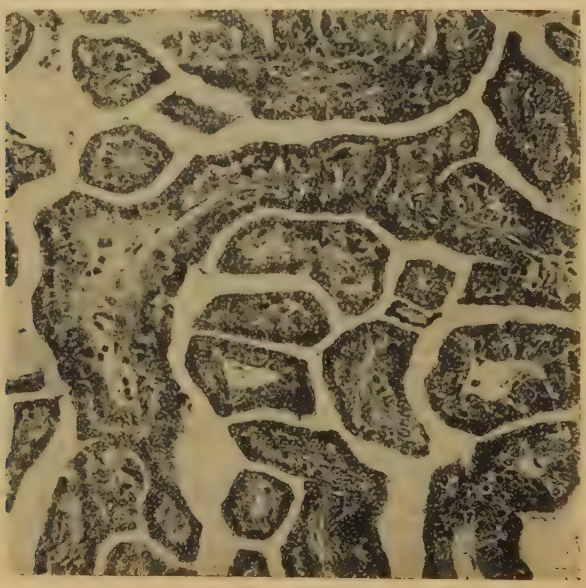

FIG. 18.
A higher magnification of part of Fig. 17. × 200.

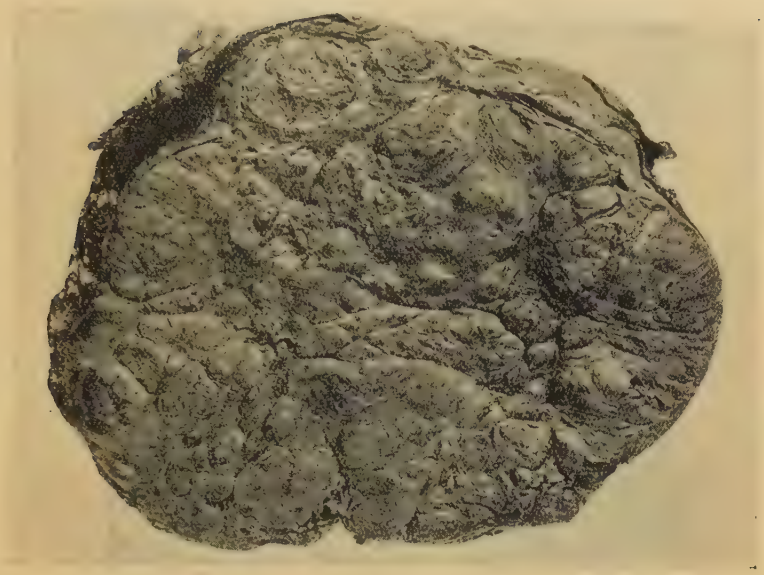

FIG. 19.

Hypernephroma No. 1, from the left kidney of a dog. Weight 4¼ lbs. Part of the left kidney is seen on the left of the tumour. The right kidney was normal. The substance of the tumour was white or yellowish white, and the firmness was less than that of the normal kidney.

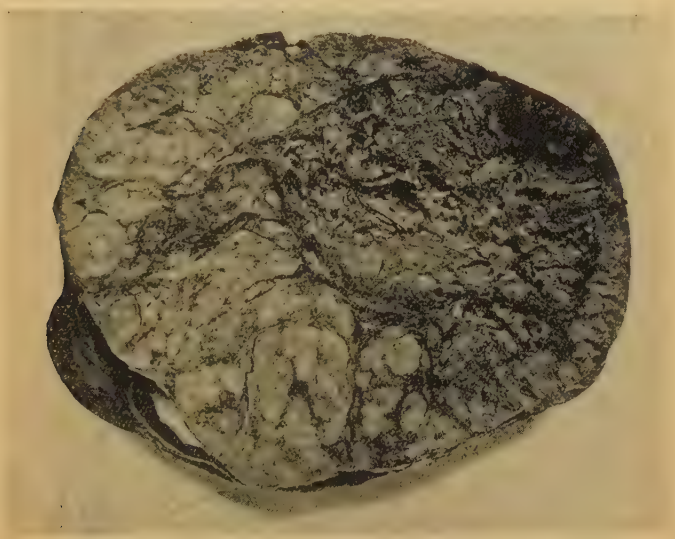

FIG. 20.

Hypernephroma No. 2, also from the left kidney of a dog. Surface exposed by section. Weight 7 lbs. A considerable part of the growth in the right half of the picture was in a state of necrosis. Part of the left kidney is seen on the left side of the tumour. The other kidney was normal.

FIG. 21.
From hypernephroma in Fig. 19. × 50.

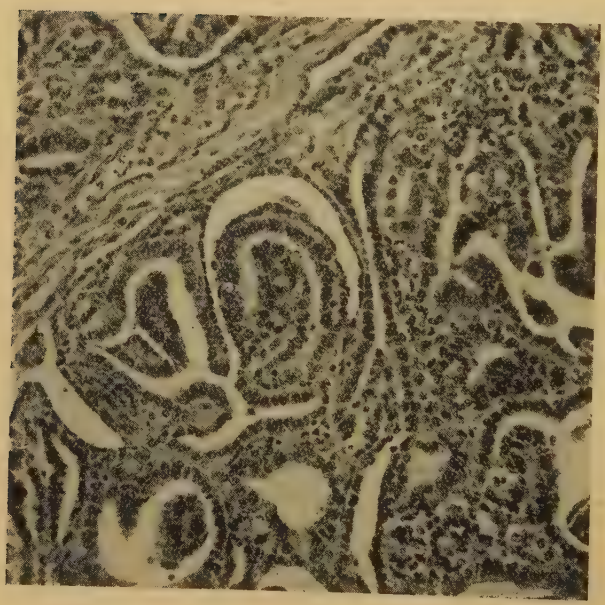

FIG. 22.
Part of Fig. 21. × 210.

FIG. 23.
From hypernephroma in Fig. 20. × 50.

FIG. 24.
From same section as Fig. 23. Shows attempts at formation of Malpighian bodies. × 300.

FIG. 25.
Metastatic tumours in lungs from the hypernephroma in Fig. 20,

FIG. 26.
From one of the metastatic tumours in Fig. 25. × 50.

Another fact of great interest is that the tumours do not contain any representatives of the branched collecting tubes of the normal kidney.*

If this interpretation of the structure of the tumours is correct they must have been derived solely from the metanephrogenic blastema, without participation of the ureteric bud which produces the collecting tubes. In other words, the tumours represent—indeed constitute—deformed kidneys, minus the medulla. It must be admitted, however, that there are difficulties in the way of denying them the status of neoplasms. The first is that they must have continued to grow long after the normal kidneys had attained their full size, and may even have been growing up to the time when the animals died ; and the second is the fact that one of them gave origin to metastases.

It will be observed that in both the hypernephromata here described (Figs. 19 and 20) the growth has a coarsely lobulated or composite appearance as if it had been built up around a number of separate centres. That has also been the case in some of the human hypernephromata.

The Papillomata.

These growths, like the carcinomata and adenomata, possess both epithelium and connective tissue, and it is the former that is the most important element.

The connective tissue forms what might be called the core of the tumour, and it is everywhere covered by the epithelium. This arrangement of the two components sharply distinguishes the papillomata from both the carcinomata and the adenomata.

The type of structure is the same as in normal papillæ of the tongue or of the rumen in ruminants. Even in the smallest of the papillomata the central core of connective tissue may be branched, and in the larger ones it is very much ramified, but all the branches of the stroma retain the surface covering of epithelium. A papilloma as a whole may be either undivided or branched, and those of large size sometimes resemble a cauliflower in consequence of repeated branching. In the pharynx and gullet of the ox they often grow in bunches.

The growths may be of any size up to the volume of a man's fist, or even larger ; but such a size is quite exceptional.

The papillomata are comparatively common tumours in the dog, ox, and horse. Their commonest sites are the skin, the mouth (in the dog), pharynx, and œsophagus (in the ox) and the prepuce (in the horse).

* That is not obvious in the micro-photographs, which indeed appear to show branching open tubes at many places. Higher magnification, however, has shown that these are of the nature of crevices without epithelial lining, or are attempts to form Bowman's capsules.

FIG. 27.
Papillomata in dog's mouth.

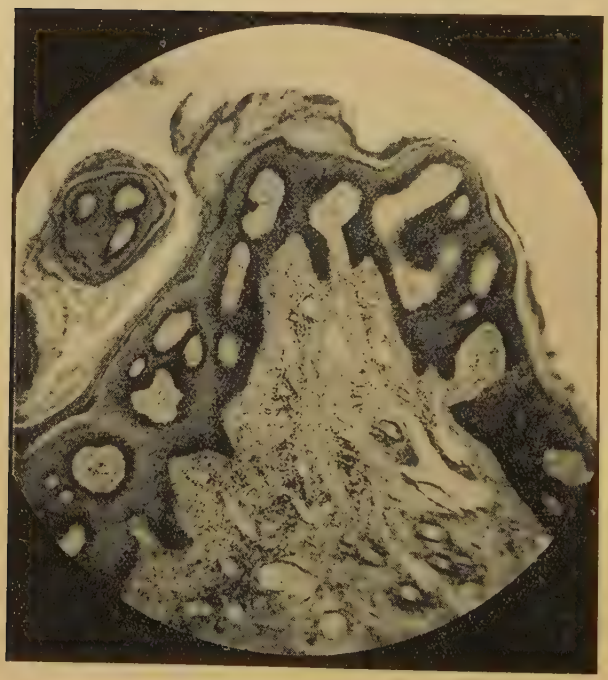

FIG. 28.
Section from a large cauliflower papilloma growing from the clitoris of a mare.

FIG. 29.
Section of a papilloma of the penis of a horse. The mass of epithelium at the upper left hand corner is part of the general surface epithelium. The groups of epithelial cells elsewhere in the stroma appear to be detached, as if the growth had taken on the characteristics of a carcinoma. × 108.

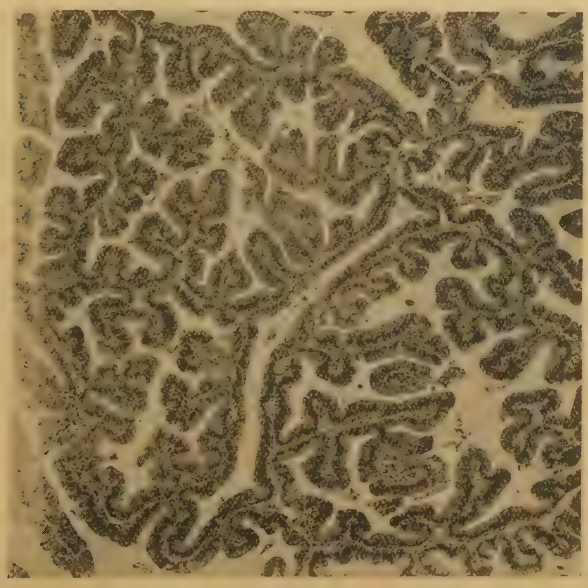

FIG. 30.
From a papilloma of the mucous membrane of the small intestine of a sheep affected with coccidiosis. Simulates an adenoma. × 50.

The cutaneous covering of papillomata is generally devoid of hair, and also of glands. In those arising in the skin the surface is usually dry, harsh, and devoid of pigment.

Microscopic examination of a small papilloma enables one to observe what has already been stated, namely, that the epithelium is superficial and does not burrow into the connective tissue part; but in large tumours this arrangement is often not obvious because of the extent to which the connective tissue core is branched. In consequence of this, parts of the epithelium may appear as islands in the connective tissue, detached from the surface growth.

The epithelium is generally less regular than in the skin or mucous membrane from which the tumour sprang; sometimes it is too luxuriant and it is often very irregular in thickness. (See Fig. 28.)

The connective tissue carries blood vessels, which may be of comparatively large size in the bigger papillomata.

The papillomata are perfectly benign tumours. Their growth is generally comparatively slow, and they never attain an enormous size. As long as they retain their ordinary type of structure they are never metastatic, but the surface epithelium of a papilloma is capable of giving rise to a carcinoma.

It appears probable that the papillomata should be excluded from the neoplasms because the orderly way in which the epithelium is arranged and the slow rate at which growth takes place do not suggest the unlimited power of multiplication which is the rule in the true neoplasms. Many of them appear to be produced by chronic irritation, and some are transmissible by inoculation.

Actual outbreaks of papillomata in the mouth have been observed in packs of fox hounds, and it has been shown that the disease can readily be transmitted from a diseased to a healthy dog by the application of a scraping from a papilloma to a small scarified area on the tongue. In many of these cases the disease comes to a natural end, the outgrowths gradually diminishing in size and eventually disappearing. Experiments have indicated that immediately after such recovery the dog cannot be infected experimentally again.

It is not improbable that the very extensive growth of papillomata which is not infrequently found in the pharynx and gullet of the ox is also due to an infection of some kind.

(*To be continued.*)

REVIEWS.

Anatomie und Pathologie der Spontanerkrankungen der kleinen Laboratoriumstiere. Herausgegeben von Rudolf Jaffé. Berlin, 1931. Verlag von Julius Springer. Price bound 102 Marks ; unbound 98 Marks.

This work extends to 832 Royal 8vo pages, and it contains 270 figures in the text. In its production over 20 authors have collaborated with Herr Jaffé, and the result is highly creditable to the ability and industry of German scientists. The work deals only with rabbits, guinea-pigs, rats, and mice, and the object set before the authors was to help experimenters who have to make use of these animals in their researches to answer a question which often arises, namely, whether anything abnormal found in an animal is actually connected with the experiment or the result of some spontaneous disease. Obviously, to find a confident answer to such a question the experimenter must have a good knowledge of the anatomy and histology of the animal with which he is dealing, and also of the diseases to which members of that species are naturally subject. It was with the object of gleaning, as far as possible, the existing knowledge on these matters that so many collaborators were employed in dealing with different parts of the subject.

In the first half of the work the chapters deal with the normal structure and the pathological anatomy of the different systems of the body. The remainder deals with bacterial and parasitic diseases, and tumours.

It must be admitted that a work constructed in this way makes it a little difficult to find the desired information, but there is a useful index at the end. It is not likely to have any competitor in view of the great cost of its production, and it will certainly prove helpful to many of those who are engaged in medical and veterinary research.

Veterinary Obstetrics. By W. L. Williams. Second Edition, 1931. Published by the Author, Ithaca, N.Y. Price 30s.

The first edition of this work appeared in 1917, and the author in referring to that in the preface to the present edition says that facts have since come to light which justify, if they do not demand, a radical departure from the traditional view. The present treatise is based upon the belief that the pathological phenomena of veterinary obstetrics are as natural as those of physiological birth, are authentically predictable, and subject to an important degree to control.

The work extends to 482 Royal 8vo pages, and it has 101 useful illustrations. The first part deals with the anatomy and physiology of the generative organs as far as these have a special bearing on obstetrics. Then follow chapters on the diagnosis of pregnancy, and the hygiene of breeding animals. The second half of the book deals with dystocia and obstetrical operations, and diseases incidental to pregnancy.

Regarding to the bacterial flora of the pregnant uterus, the author, after referring to the great number of different organisms that have been cultivated from the genital passages of the domesticated animals, says, " The presence of various types of bacteria is perfectly consistent with ideal reproductive health. The bacteria probably vary according to geographic and climatic location. They vary according to species of animal, and the type probably

varies according to the state of gestation. *Bacillus abortus* appears to be the most prevalent of all bacteria in cows aborting in late pregnancy, but it is rarely recognised in early abortions." The *B. abortus* ought to be pleased to find itself in such a respectable company.

Bainbridge and Menzies' Essentials of Physiology. Seventh Edition, edited by H. Hartridge. London, Longmans, Green and Co. Price 14s. net.

The first edition of this work appeared in 1914, and the fact that a seventh edition has already become necessary affords strong evidence that it is well adapted to the requirements of students of medicine.

It seems to strike a reasonable balance between the two extremes in books of this class, of being too elementary or too exhaustive for the purpose of medical undergraduates.

ABSTRACTS.

XANTHIN CALCULI IN SHEEP.

By Easterfield, Rigg, Askew and Bruce.

According to the authors, xanthin (2·6 dioxypurin) has been recognised for more than a century as a rare constituent of urinary calculi. It is present in human urine and the amount is increased during inflammation of the kidneys. Its presence in the urine of sheep and of pigs was recognised at an early date. It occurs in the blood, muscles, and many of the glands of animals, and is widely distributed in the vegetable kingdom. This common occurrence is not surprising since xanthin is a regular though secondary product of the hydrolysis of nuclein. Owing probably to the action of xanthin oxidase in the liver and other organs, the quantity of xanthin in urine and blood under normal conditions is very small, and concretions of this compound are regarded as almost the rarest of urinary calculi.

During the course of an investigation at the Cawthron Institute into the mineral contents of Nelson pastures, the occurrence of stone in the kidneys of a sheep was pointed out by several farmers in different parts of the Waimea County. Further inquiry and the careful examination of a number of kidneys showed that on some farms kidney calculi were very prevalent; that in every case the calculi consisted of xanthin; and that serious loss of stock from calculus trouble was restricted to farms on one particular soil type which was of low fertility.

These facts suggested that the abundant occurrence of xanthin calculi might be caused by deficiencies in the pastures associated with the soil type in question, and the analyses in connection with a comprehensive soil survey of the Nelson district by the agricultural division of the Cawthorn Institute had already shown that the soil was very deficient in lime and phosphoric acid.

All the calculi which have come to the notice of the authors have been taken from stock raised on soil of the Moutere Hills type, which covers an area of 300-400 square miles. Cases have been seen or reported from eight districts, but the occurrence has been most striking in one valley, in which on one small farm, grazing 120 sheep, 10 sheep were found in six months with kidney calculi as a predisposing cause of death. The owner picked out the badly affected sheep by their appearance and killed two sheep in the presence of the authors to demonstrate this. In both cases the kidneys were badly affected.

No instance of calculi trouble has been reported in the case of sheep reared on the more fertile soils of the Waimea County, and on the few areas of Moutere Hill soil where, by lime and phosphate treatment, good pastures have been established, the presence of calculi in the kidneys of sheep has likewise not been recorded.

Though the calculi generally consisted of a gravel made up of a mixture of large and small stones with particles as fine as dust, a calculus from Harakeke represented a complete cast of the enlarged kidney pelvis. The specimen weighed 36 gm. Small stones were frequently found in hoggets which were in sufficiently good condition to warrant their slaughter for meat. Large stones may occur in both hoggets and full-mouthed sheep, and wethers and ewes seem equally affected.

Though the calculi from the sheep on the Moutere soil are of a light grey stony appearance suggestive of calcium carbonate or phosphate, they

were proved in every case to be composed of xanthin, $C_5H_4N_4O_2$, which is remarkable since xanthin calculi are regarded as extremely rare.

In a particular case the dried calculus left only 0·1 per cent. of ash on ignition. It contained 35·35 per cent. of nitrogen, which is surprisingly near to the value 36·8 per cent. required for pure xanthin. After purification by solution in ammonia, decolourisation with animal charcoal, and reprecipitation by spontaneous evaporation of the ammonia, the xanthin was obtained in characteristic microscopic globules containing 36·6 per cent. of nitrogen, thus agreeing well with the calculated value.

The xanthin showed the following qualitative reactions which confirm its identity: It dissolved in ammonia and more readily in caustic soda and was reprecipitated by dilute acetic acid. Evaporated to dryness with nitric acid, it left a yellow residue which gave a yellow solution with ammonia and deeper yellow with caustic soda. The soda solution became purple-violet when gently warmed. Evaporated to dryness with hydrochloric acid and chlorate of potash it left a residue which turned violet with ammonia. The solution in baryta water upon treatment with diazotised sulphanilic acid yielded a brick-red precipitate.

Symptoms.—Affected sheep are frequently in poor condition. In a number of cases, there was no fat surrounding the kidneys. Two 2-toothed sheep with large calculi in the kidneys had a live weight of 49 lb. and 52 lb. respectively. Healthy sheep of the same age should weigh not less than 80 lb. In severe cases, the sheep mope around the paddocks and show little inclination to graze. Frequently, such sheep have arched backs with the hind-legs drawn under the body. There appears to be great difficulty in urination and the sheep may be seen straining frequently. One sheep badly affected was distinctly anæmic in appearance. In severe cases, one or both kidneys may be greatly enlarged and full of calculi. Sometines, rupture of the kidney takes place and liquid accumulates in the body cavity quickly causing death.

Stock Conditions.—On many parts of the soil of the Moutere Hills type, the results obtained by farmers from stock—both sheep and cattle—compare unfavourably with those obtained in other parts of the Nelson district where the soils possess higher fertility. They included the poorer results associated with stock on Moutere Hills country—low lambing returns, greater mortality among sheep and cattle, inability to fatten lambs or hoggets on any pastures on the Moutere type of soil unless lime and phosphate treatment had been given to the land or supplementary feeding is undertaken, comparatively low wool returns, etc. Many instances were reported where stock had eaten earth and chewed bones and sticks. Mixtures of bonemeal and salt were eaten greedily by both cattle and sheep on several farms where a poor condition of stock was noticeable. Great improvement in the condition of the stock had resulted from bonemeal feeding.

Chemical analyses showed a striking resemblance between the Moutere Hills soil and that of two other areas on which the occurrence of xanthin calculi had not been observed, but perhaps overlooked. All three were highly deficient in available and total phosphoric.

The pasture on the Moutere Hills was found to have a low content of nitrogen, soluble ash and lime phosphate, and iron and manganese. The autumn samples had a low iron and a high manganese content, but in spring samples the percentage of these were normal. It therefore appeared that a deficiency in iron was not the main cause of the trouble. (*Journal of Agricultural Science*, Vol. XIX, Part III, 1929.)

FOOT-MOUTH DISEASE.*

Survival of the Virus.—Experiments bearing on this question showed that the virus in epithelium, at 1° C., was not noticeably affected after 77 days at *p*H 7·0 to 8·0, or in 93 days at *p*H 6·4 to 6·8. At each laboratory temperature, 12° to 20° C. there was relatively rapid but irregular destruction of the virus in solutions of *p*H 6·4 to 7. The survival was longest in solutions of *p*H 7·2 to 8·0, with the optimum at 7·4; in phosphate solutions of *p*H 7·4 survivals of 71 days and 73 days were noted.

Striking features in the experiments were the long periods of survival on leather, hides, and rubber (80 days, 89 days, and 102 days respectively).

In experiments with virus in epithelium on limed wall the virus survived for 27 days, and for two to three weeks on wood, glass, and brick. On stockinette and hessian, which are materials used for wrapping imported meat, the virus survived for 45 days in one experiment and 46 days in another at 12° to 20° C. On the same materials there was survival for 47 days at minus 2° to plus 2° C. (28·4° to 35·6° F.).

In experiments bearing on survival of the virus in bovine carcases the animals were infected naturally with the disease by contact with active cases, and their reactions were followed very closely. They were slaughtered at different stages of the disease, and especially at an early stage when the temperature was rising, but it proved to be difficult to judge accurately the best time for slaughter in order to insure the presence of the virus in fairly high concentration in the blood and organs. In every case the carcase was kept at " chilling " temperature, 29° F., but, as in the imported-meat trade, the " offals " were frozen at 15° to 18° F. Samples were tested on swine, generally by I.M. inoculation, and the following periods of survival were recorded :—

Up to 33 days in tongue, cheek, and the washings from an intact quarter. Up to 40 days at 29° F. in meat wrapping cloth soaked in virulent blood. Up to 80 days at 29° F. in bone-marrow and in blood-clot.

In the Second Progress Report it was stated that the virus was found to survive as long as 42 days in the bone-marrow of a " chilled " carcase, and 76 days in " frozen " carcase. The survival in " chilled " carcases in these later experiments was thus almost twice as long as that mentioned in the earlier Report.

In some of the recent experiments the virus which was employed was found to be irregular and uncertain in its effects, but nevertheless sufficient evidence of survival for long periods was obtained to emphasise the dangers that may be associated with " chilled " and " frozen " beef.

Hides removed from the slaughtered animals were salted and stored in an ordinary loose-box, the temperature of which differed little from that of the outer air. In one instance such a hide was found to be still infective to pigs after 46 days.

Disinfection.—Sodium carbonate in the form of ordinary washing soda was found to be a most useful and reliable agent. In order to destroy the virus within one hour it was necessary to employ a 1 per cent. solution, but a 4 per cent. solution at room temperature practically always killed the virus within 15 minutes, and at 50° or 60° it was effective within five minutes. As commonly used in butchers' shops and abattoirs for cleansing

* From the Fourth Progress Report of the Foot-Mouth Disease Research Committee, 1931, published by H.M. Stationery Office, London. Price 7s, 6d. net.

chopping blocks, &c., washing soda therefore appears to be very reliable as a virulicidal agent.

Lime wash was found to be quite effective in destroying the virus if used in the wet state and brought into contact with it. It was found, however, that virus may survive for 27 days on a dry limed wall.

Types of the Virus Derived from British Outbreaks.—During the period ending June, 1930, an attempt was made to procure virus from all the outbreaks occurring naturally in Great Britain, and to assign each strain to its immunological type. Altogether 23 British strains were examined and 21 of them proved to be " O " strains. No " A " or " C " strains were encountered, and the two remaining strains could not be assigned to any recognised type. They were not of intermediate type, and immunologically they were quite distinct.

It was also found that these two viruses are themselves quite distinct immunologically from each other.

Adding the results of similar previous tests of the viruses concerned in British outbreaks, 40 British strains have now been examined, and of that number 37, or over 92 per cent., have been proved to be " O " strains.

In September, 1928, ten samples of infected material were received from Buenos Ayres. These samples could not be regarded as in any way furnishing an accurate idea of prevalence and distribution of the types in Argentina. Nine of the strains were obtained from animals which had been in one particular market during the period of a few days, and they might well have represented nine cases of the same outbreak.

The strains were first tested in cattle, and it was then found that only three were of the " O " type. The three strains assigned on bovine tests to the " O " type were adapted to the guinea-pig, and in that test two of them were shown to be characteristic " O " viruses. The third strain was apparently a weak or atypical " O " strain, which induced reaction with generalisation in a large proportion of the guinea-pigs immune to " A " and " C " viruses, but also set up generalisation in a small proportion of the " O " immunes.

Of the seven viruses which, in the ox, had overcome an " O " immunity, one could not be adapted to the guinea-pig. The remaining six were satisfactorily adapted and were found not to belong to any recognised type. As with British viruses 71 and 72, they induced primary reaction and generalisation in the majority of the " O," " A," and " C " immune guinea-pigs.

The proof of the occurrence, both in Great Britain and in South America, of viruses which do not conform to one of the three known types is of considerable importance in relation to the question of artificial immunisation.

The Disease in Rodents.—In 1929, a Veterinary Inspector of the Ministry of Agriculture, when dealing with an infected farm, noticed that many rats on the premises were lame, and four were forwarded to the Ministry. Material from one of these rats, when inoculated into guinea-pigs, caused a local (primary) reaction followed by generalisation, but it was not found possible to carry on the infection to other guinea-pigs.

About the same time a rat was trapped at the Research Committee's Experimental Station, and inoculations of suitable tissues into guinea-pigs were made, although the rat exhibited no lesions that could be regarded as characteristic or even definitely suspicious. The guinea-pigs developed characteristically primary and secondary vesicles, and the virus was readily carried on in the guinea-pig in series.

After the third passage sufficient lymph was obtained to allow the demonstration of the passage of the virus through a Seitz filter. The feeding of carcases of infected guinea-pigs to three swine caused characteristic attacks of foot-mouth disease, and it was later proved that the virus (S.I.) belongs to the common " O " type.

The virus S.I. has also been carried on in a series of rats, and the most reliable method of infection was found to be simultaneous intradermal and intramuscular inoculation. The best results were obtained with well grown adult rats.

The vesicles are small and often difficult to appreciate, and a point of interest is the great frequency with which small vesicles form on the tail between the hair follicles. Successful attempts were made to transmit the infection from rat to rat by ingestion (of rat carcases), about half the animals developing the disease. The infection was also conveyed from rat to rat by contact, and 33 rats were infected in that way. In contact cases inoculation showed the tail to be the region in which the presence of virus could most frequently be proved ; this fact is probably related to the great exposure of the tail to trauma, but it is uncertain whether the tail lesions in contact cases are primary or secondary.

Four other strains of virus were inoculated into rats. The Research Committee's Virus I. (" O " type) was almost as virulent for rats as the naturally occurring virus S.I., but the other three strains were very definitely less potent, although they induced characteristic vesicles in some of the inoculated animals.

White rats were readily infected by inoculations (I.D. plus I.M.) with virus I. and S.I., and the infection was also transmitted by ingestion of infected carcases ; but infection by contact was definitely less successful than with wild rats.

Experiments failed to infect house mice ; but after some failures white mice were successfully infected, although the lesions were poor.

The hamster was shown to be susceptible by inoculation.

The Report adds that the incidence of the disease in the field lends little or no support to the suggestion that rats or other rodents play any important rôle in its dissemination.

The Report contains a series of excellent plates showing the lesions in the common rat.

The Disease in the Rabbit and Hedgehog.—Nine wild rabbits, not fully grown, were obtained and two of them (Nos. 1 and 2) were inoculated intradermally on the tongue with 0·2 c.c. of fresh 1 in 50 virus strain G.F., and 1·0 c.c. was given at the same time I.M. One of the inoculated rabbits was found dead the next day. The other six rabbits were placed in the same box, and all the animals, when they were observed, kept huddled close together.

Rabbits 1 and 2 became infected and small vesicles appeared on the tongue at the sites of inoculation, and in Rabbit 1 some very small vesicles appeared on the third day on the gums and lips. The lesions were slight, and in none of the six other rabbits could any foot-mouth lesions be detected, although they were examined daily for twelve days.

The experiments suggested that wild rabbits are not particularly susceptible to the disease.

The fact that foot-mouth disease is transmissible to the hedgehog was reported in the Second Progress Report of the Committee, and later experiments have brought to light interesting facts with regard to the disease in that animal. They show that the hedgehog is highly susceptible to infection

with the " O " type of virus, and the disease seems to be more fatal than in any other species of animals that is known to be attacked. Virus obtained directly from the guinea-pig is not of very high virulence for the hedgehog. It sometimes produces infection by feeding. When inoculated in large doses it sets up, however, in most cases, a fatal disease, but a small proportion of animals may recover. The most effective method of inoculation is intradermally, or by scarification on the sole of the heel or above it : intramuscular inoculation has less certain results.

By intradermal passage with vesicle fluid taken at the earliest stage of the disease the virus rapidly becomes adapted to the hedgehog so as to produce severe uniformly fatal disease which spreads rapidly from the infected animal to healthy animals kept in contact with it.

Hedgehogs infected from virus taken direct from the guinea-pig appear to be less certainly infective for healthy hedgehogs, and only some of those placed in contact become infected. The disease which is contracted is really mild ; it may be undetected clinically and the animals may recover.

The severe disease in hedgehogs, produced by virus which has been adapted to the species, spreads naturally from the inoculated to the healthy animals with remarkable regularity, producing generalisation in three to six days and death in from nine to fourteen days after first exposure. The disease continues to spread among fresh hedgehogs exposed to infection provided they are placed in contact with diseased animals at an early period of their attack.

The macroscopic lesions in the acute form of the disease are characteristic, and are almost invariably confined to the epidermis covering the pads, and in the buccal, genital, and perineal regions.

The epidermal lesions vary in gravity with the severity of the disease. Sometimes the animals die before there are visible or pronounced changes in the epidermis, and in these cases the virus may be demonstrated in the internal organs (blood stream or bone-marrow).

In the occult form of the disease the animal may recover or it may die, and the virus may be demonstrated after death in the apparently healthy pad epithelium or in the bone-marrow.

In the hyper-acute form of the disease contracted naturally by contact the virus is present in high concentration in the characteristic epidermal lesions at an early stage of the disease, but usually cannot be demonstrated in the epithelium or marrow at the time of death.

Diseased hedgehogs may spread infection to guinea-pigs kept in contact with them, probably chiefly through wounds on the feet ; but hedgehogs do not appear to become infected by contact with infected guinea-pigs.

There has been no evidence of change in type of the virus after passage through the hedgehog.

A pen which has contained several infected hedgehogs among which the disease was spreading by contact may not be infective for fresh hedgehogs when the infected hedgehogs are removed.

The resistance of hedgehogs to foot-mouth disease can be increased by I.M. inoculation with formalised virus, but the degree of protection is relative and is not adequate to prevent a fatal infection from contact with hedgehogs suffering from the hyper-acute form of the disease.

No evidence has been obtained that a hedgehog can recover and become a carrier and a source of infection although apparently in a healthy condition. The only carefully observed cases in which the disease has spread by contact have been cases of severe overt infection. The disease usually ends in death,

and the occasional animals that recover do not appear to be a source of infection.

Duration of Immunity in Cattle.—In a previous Report it was noted that animals had resisted intramuscular inoculation of virus 13½ months after infection. The present Report records further experiments and observations with regard to the duration of immunity. The most extensive observations were made on a series of animals which reacted to foot-mouth disease virus at the Experimental Station at Pirbright, and which were afterwards confined to the closed premises there and carefully protected from any re-infection until their immunity was tested. Several different strains of virus were used, both for inducing the first reaction and testing the immunity, but in all cases they were of the " O " type. The first reaction was, in most cases, set up by intramuscular inoculation of virus. A third of the animals contracted the disease naturally by contact, and no evidence was found to suggest that the degree or duration of the resulting immunity was influenced in any way by that factor.

In every instance the humoral immunity was tested by intramuscular inoculation of virus, and the activity of the virus was always assured by suitable controls. In the main experiment under this head 18 cattle were tested for humoral immunity at periods varying from 13 months to 32 months (in two cases) after the first infection, and in no case did an attack of the disease result. One of the cattle tested after 13½ months gave a slight temperature reaction, but there were no other signs of systematic disturbance, and no evidence whatever of vesicle formation. Moreover, the blood of this animal, taken at the height of the febrile action, failed to infect susceptible guinea-pigs.

In addition to these special tests, many cattle that were " O " immune were inoculated with " O " strains of virus. These inoculations, performed in connection with other experiments, indicated that the period of immunity varied from three weeks to a year. In no case at Pirbright has an intramuscular inoculation overcome an " O " immunity with the virus of the same type, and in tests over periods up to 32 months it has been impossible to demonstrate any loss or decline of humoral immunity.

Chemotherapeutic Experiments.—Since from time to time reports have appeared to the effect that particular chemical substances have a prophylactic action or a curative effect, or at least a modifying effect on the severity of the infection with the virus of foot-mouth disease, a total of 90 different chemical substances were tested.

In any instance where the product seemed to have some action, suggested by the non-appearance of lesions in the treated animal after infection, further and more exhaustive tests were made. Special attention was given to such substances as acriflavine, trypanblue, bismuth salts, intramine, &c., but none of the substances was found to definitely modify the course of the infection when given in non toxic doses.

Survival of the Virus in Milk.—It was found that virus can survive in fresh milk which has been " chilled " immediately after being drawn from the cow and subsequently kept at a low temperature (4° C.) for about 15 days. If the milk, subsequent to being " chilled," is kept at a higher temperature (18° C. or about 60° F.) the virus will survive in it for about six days. After that, at this temperature, the milk may become acid, and this acidity inactivates the virus.

When the virus is added to milk which has been sterilised by steam it can remain viable in that medium from 30 to 35 days at room temperature.

It was found that when milk containing virus is exposed in a thin layer to high temperature it is very quickly rendered non-infective ; five seconds at 65° C. is sufficient to destroy the virus, although $2\frac{1}{2}$ seconds fails to do so. In relation to this result the Report points out that commercial drying is not effected by heat alone, but by the simultaneous use of air currents or reduced pressure, and that milk which is being dried is evaporating at the same time as it is being subjected to heat, so that the temperature to which it is exposed must be lower than that of the Drying Chamber until all the water has been removed.